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Bridge water mediates nevirapine binding to wild type and Y181C HIV-1 reverse transcriptase—Evidence from molecular dynamics simulations and MM-PBSA calculations

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ABSTRACT

The important role of the bridge water molecule in the binding of HIV-1 reverse transcriptase (RT) inhibitor complex was elucidated by molecular dynamics (MD) simulations using an MM-PBSA approach. Binding free energies and thermodynamic property differences for nevirapine bound to wild type and Y181C HIV-1 reverse transcriptase were investigated, and the results were compared with available experimental data. MD simulations over 3 ns revealed that the bridge water formed three characteristic hydrogen bonds to nevirapine and two residues, His235 and Leu234, in the binding pocket. The energetic derived model, which was determined from the consecutive addition of a water molecule, confirmed that only the contribution from the bridge water was essential in the binding configuration. Including this bridge water in the MM-PBSA calculations reoriented the binding energies from −32.20 to −37.65 kcal/mol and −28.07 to −29.82 kcal/mol in the wild type and Y181C HIV-1 RT, respectively. From the attractive interactions via the bridge water, His235 and Leu234 became major contributions. We found that the bridge water is the key in stabilizing the bound complex; however, in the Y181C RT complex this bridge water showed weaker hydrogen bond formation, lack of attractive force to nevirapine and lack of binding efficiency, leading to the failure of nevirapine against the Y181C HIV-1 RT. Moreover, the dynamics of Val179, Tyr181Cys, Gly190 and Leu234 in the binding pocket showed additional attractive energetic contributions in helping nevirapine binding. These findings that the presence of a water molecule in the hydrophobic binding site plays an important role are a step towards a quantitative understanding of the character of bridge water in enzyme-inhibitor binding. This can be helpful in developing designs for novel non-nucleoside HIV-1 RT inhibitors active against the mutant enzyme.

1. Introduction

Reverse transcriptase (RT) is a key enzyme in the replication cycle of the human immunodeficiency virus type 1 (HIV-1), catalyzing the conversion of virally encoded RNA into proviral DNA [1]. This essential step in the retroviral life cycle is targeted by a variety of drugs in clinical use to combat AIDS (acquired immune deficiency syndrome). RT is a heterodimeric enzyme with subunits of 66 and 51 kDa. The p66 subunit consists of fingers, palm and thumb subdomains (named for their resemblance to a right hand), as well as connection and ribonuclease H (RNase H) subdomains.

There are two main classes of RT inhibitors [2]. The first class consists of nucleoside analogues (nucleoside reverse transcriptase inhibitors, or NRTIs) such as AZT (3′-azido-3′-deoxythymidine), ddI (dideoxymosine) and ddC (dideoxycytidine). These are competitive inhibitors of the nucleotide substrate, and bind to the polymerase active site upon metabolic activation. After incorporation in the DNA strand instead of dNTP they cause premature termination of the newly synthesized chain. In addition, NRTIs also act on other host DNA polymerases, which explains their toxicity [3]. The second class consists of non-nucleoside inhibitors (NNRTIs) such as HEPT (1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine) [4], TIBO (tetrahydroimidazo-[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one) [5], nevirapine (dipyridodiazepinones) [6], and efavirenz ((−)-6-chloro-4-cyclopropyl ethynyl-4-trifluoromethyl-1,4-dihydro-2H-3,1-benzoxazin-2-one) [7]. These inhibitors are highly specific for HIV-1 RT and lock it into an inactive conformation by fitting into an allosteric site approximately 10 Å from the polymerase active site, causing a displacement of the catalytic aspartate residues. Furthermore, they show lower cellular toxicity than NRTIs.

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Reported crystal structures of RT include those of unliganded RT [8], RT complexed to dsDNA [9], RT bound to several NNRTIs [10,11], and RT complexed to dsDNA and a deoxynucleoside triphosphate (RT/dsDNA/dNTP) [12]. The non-nucleoside binding pocket only exists in the structures of RT complexed to a NNRTI, with its formation probably being induced by the proximity of the inhibitor. In unliganded RT, the p66 thumb subdomain is folded into the DNA-binding cleft and lies over the palm subdomain, nearly touching the fingers subdomain in a "thumb down" configuration. As a consequence, the DNA-binding cleft is closed. Moreover, the conformational change effected by NNRTI binding reduces the catalytic efficiency of the enzyme [13].

The rapidity of the selection of drug-resistant HIV in patients was such that single-point mutations in the virus made first-generation NNRTIs such as nevirapine unusable in monotherapy [14]. Among the mutations in RT that were originally described for nevirapine resistance were those at Tyr181 and Tyr188, both of which gave rise to high-level resistance [15]. The mutation of tyrosine at position 181 has frequently occurred, not only when nevirapine was treated but also in many other NNRTIs, and the change is almost always to cysteine. In the case of the mutation of tyrosine at position 188, a variety of mutation was reported. Nevirapine and HEPT select the Tyr188Cys mutation, whereas TIBO or α-APA result in Tyr188His or Tyr188Leu mutations [16]. Experimental results show that the non-nucleoside inhibitors lose their inhibitory efficiency by 20- to 1,000-fold when the mutation occurs in the HIV-1 RT binding pocket [17].

Computational modeling studies on HIV-1 RT and nevirapine have been performed for a few decades to gain more understanding at the molecular level. The conformational analysis of nevirapine by quantum calculation revealed that the cyclopropyl group is rotatable, but the minima appeared at 218° [18]. The basic postulate Gaussian network model (GNM) revealed that the p66 thumb's mobility was extremely sensitive when HIV-1 RT was bound to nevirapine. Collective motions analysis showed the key residues, Leu100, Trp229 and Leu234, covered nevirapine inside the binding pocket [19]. High-level quantum mechanical (QM) theory, implemented through the ONIOM approach, revealed weak π–π and H–π attractive interaction between Tyr181 and nevirapine [20,21]. Although the QM studies provide accurate interaction data according to theory, the studies based on rigid structures still lack information about the dynamics of binding in the RT-nevirapine bound complex. Thus, the simulations approach has also been applied to investigate the properties, interactions and binding energy of the reverse transcriptase complex to non-nucleoside inhibitors, in order to handle larger systems than can be accommodated by QM techniques [22]. The conformational changes of the RT pocket have been investigated through molecular dynamics (MD) simulations. Specific demonstration of the dihedral angle rotation of N-CA-CB-CG in Y181 showed the induced effect from mutation [23]. However, the interrelationship of nevirapine and the Y181C mutation also needs some proof from energetic prediction to be linked with the experimental affinity. The combined Monte Carlo (MC) simulations with free energy perturbation (FEP) revealed a fold resistance energy of 3.88 ± 0.3 kcal/mol from nevirapine relatively to efavirenz [15], and the effectiveness of nevirapine against the Y181C mutated form of the enzyme versus the wild type [24]. Ways to calculate the absolute binding free energy between HIV-1 RT and nevirapine have been developing since first being introduced with the QSAR correlation [25]. One of the most useful methods is the Molecular Mechanics/Poisson–Boltzmann Surface Area (MM-PBSA) method [26] because it is based on a compromise between speed and accuracy in the calculations. The combined MD/MM-PBSA method could explain the lost activity of nevirapine when the Y181C mutation occurs from the change in free energy by −5.94 kcal/mol [27]. Currently, numerous computational studies have shown quantitative agreement between calculated and experimental binding affinities in the HIV-1 RT system. However, the role of solvent molecules in the binding mechanism between the hydrophobic pocket and nevirapine is still of interest for further study.

Therefore, the main objective of this work was to study solvent molecules inside the hydrophobic binding pocket and introduce the key step in the MM-PBSA calculations when it was essential to include explicitly the solvent. The roles of the water molecule in HIV-1 RT/nevirapine complex have been reported previously by Rizzo and co-worker [28]. In this study we investigated a different water molecule, the bridge water molecule (WAT1067), which is located around the oxygen of nevirapine. A combination of molecular dynamics simulations and MM-PBSA calculations (MD/MM-PBSA) was applied to study the wild type and the Y181C HIV-1 RT/nevirapine complexes in order to obtain the theoretical binding energy, and observe the dynamics of the RT pocket. The motion and role of water inside the binding pocket were also investigated. The results will be useful in determining the molecular level of HIV-1 RT/nevirapine interaction in solution, and as guidance for other NNRTIs which have similar binding to nevirapine. This basic information can be used in the development of higher potency NNRTIs against mutant enzymes.

2. Computational methods

2.1. Molecular dynamics simulations

Model structures of the wild type and the Y181C HIV-1 RT complexes with nevirapine were constructed based on the crystallographic code 1VRT [29]. The enzymes were mutated at position 181 from tyrosine to cysteine by the SPdbV3.7 program [49]. After all missing residues were added, the mutant model was then minimized using 5,000 steps of steepest descent, and then switched to the conjugate gradient algorithm in the Sander module of the AMBER program package in order to remove bad steric interactions. Nevirapine was firstly optimized at the B3LYP/6-31G(d,p) level. Then the electrostatic potential that surrounds nevirapine was calculated by a single-point calculation at the HF/6-31G(d) level in GAUSSIAN98 [30]. The electrostatic potential was fitted into the partial atomic charge of nevirapine by the RESP [31,32] charge method. Preparation of the force field parameters of nevirapine was done by using the Antechamber module [33] of AMBER.

Molecular dynamics simulations were performed using an AMBER7 [34] program with an AMBER 1999 force field [35]. Each complex system was immersed in an octahedral box of 10 Å from the solute surface using TIP3P water [36]. A total of 42,170 solvent molecules were generated followed by 7 Cl^- ions for neutralization, resulting in a total of 143,900 atoms in the system. Periodic boundary conditions and constant temperature and pressure were used. The non-bonded cutoff distance was set to 11.5 Å. The integration time step was 2 fs, with SHAKE [37] applied to constrain the bonds involving hydrogen atoms. The restraint force on the whole complex was slowly decreased from 4.0 kcal/mol to zero during the equilibration, and simulations were run for 1 ns at 300 K. Coordinates were saved every 1 ps. After the entire HIV-1 RT/nevirapine system had been simulated for 1 ns, the low energy structure at the equilibrium was selected as the next starting structure. In order to speed up the calculation, the structure of the enzyme complexes was reduced to a smaller model in which protein residues and water molecules outside 30 Å of the mass center of nevirapine were removed. Continuation of the simulations was performed by using the AMBER9 [38] program with the Duan et al. (2003) force field [39,40] which provided a better force field for proteins. The model was set up as follows: all water

molecules inside the allosteric binding site were kept in their positions, two magnesium ions were added to the active site region, and a 10 Å spherical water cap centered on nevirapine was added near the binding site [28,41]. The simulations were carried out for 3 ns at 300 K under constant volume periodic boundary conditions. Simulations were performed on a 2.4 GHz, 514 Mbyte system running on a Linux7.3 PC.

2.2. MM-PBSA analysis

All water molecules and ions were removed before the continuum solvent model was applied to calculate the MM-PBSA energies. The electrostatic contribution to the solvation free energy was calculated by the Poisson–Boltzmann method (PB). The hydrophobic contribution to the solvation free energy was determined using a solvent-accessible surface area (SA) dependent nonpolar solvation term. The description of the process to obtain the MM-PBSA energies derived from the thermodynamic cycle can be found in Wang et al. [33]. In this work, snapshots of 1.5–3 ns were sampled. In total, 500 snapshot structures were used in the MM-PBSA calculations and analysis. To investigation the effect of the explicit solvent water molecule, we included the closest water – one, two, and three – molecules to extend the MM-PBSA calculations.

2.3. Theoretical background

The experimental binding affinities of nevirapine with HIV-1 RT were obtained from enzymatic kinetic studies, which were represented variously by: K_d (the equilibrium dissociation constants); IC_{50} (50% inhibitory concentration of nevirapine against HIV-1 RT); or EC_{50} (50% effective concentration, or concentration required to protect cells against the HIV cytopathogenicity by 50%). These experimental data can be converted to the binding free energy between nevirapine and HIV-1 RT by using the thermodynamic equation ($\Delta G \sim RT \ln$(activity) in kcal/mol) [28].

To find the binding energies from the theoretical calculation, two strategies in the MM-PBSA calculations involving the explicit water molecule were derived. First, the MM-PBSA energies of individual solutes, including the explicit water molecule, were calculated. Then each individual energy term, which included the energy of the explicit water molecule, was subtracted from the free energy of the complex. The binding free energies ($\Delta G_{binding}$) were obtained as shown in Eq. (1):

$$\Delta G_{binding} = \Delta G_{complex} - (\Delta G_{enzyme} + \Delta G_{Nevirapine} + \Delta G_{WAT}) \quad (1)$$

Secondly, the appearance of the explicit water molecule could be considered as a part of the receptor. This alternative approach kept the concept that the binding energies were calculated from two parts instead of the three components. The effect from the numbers of explicit water molecules were simultaneously embedded inside the enzyme. Thus, the binding free energies ($\Delta G_{binding}$) could be obtained from Eq. (2), which is the standard MM-PBSA approach for including selected solvent molecules:

$$\Delta G_{binding} = \Delta G_{complex} - (\Delta G_{enzyme\text{-}WAT} + \Delta G_{Nevirapine}) \quad (2)$$

Entropy of the system was calculated through the normal mode analysis for a temperature of 298.15 K and 1.0 atm pressure. The total entropies for translation, rotation and vibration were obtained and converted to energy (kcal/mol) or heat capacity (cal/mol K). The $\Delta S_{binding}$ was obtained from the $\Delta S_{complex}$ in the same fashion as in Eq. (1) or Eq. (2).

An alternative way to compare the calculated result with the experimental data is to carry out relative measurements between the wild type and the mutant type enzymes. The relative activity of nevirapine against the wild type and the Y181C HIV-1 RT from experiment can be measured in terms of the fold resistance and the relative fold resistance energy ($\Delta\Delta G_{fold\ resistance} = \Delta G_{mutant} - \Delta G_{wild\ type}$) [42].

Table 1
Experimental binding affinities (μM) and energies (kcal/mol) from the wild type and Y181C HIV-1 RT complexes with nevirapine.

	Wild type HIV-1 RT/nevirapine		Y181C HIV-1 RT/nevirapine	
	Affinities (μM)	Energy (kcal/mol)	Affinities (μM)	Energy (kcal/mol)
1. K_d [45]	0.025 ± 0.010	−10.37	11.700 ± 4.30	−6.73
2. K_d [45]	0.019 ± 0.004	−10.53	2.500 ± 1.30	−7.64
3. IC_{50} [46]	0.060	−9.85	3.200	−7.50
4. IC_{50} [47]	0.032	−10.22	10.000	−6.82
5. EC_{50} [48]	0.044 ± 0.010	−10.04	3.040 ± 1.42	−7.53
6. IC_{50} [28]	0.084	−9.65	(Not reported)	

$\Delta G = RT \ln$[Activity] in kcal/mol, where ΔG represents the binding free energy (kcal/mol) with activities expressed in units of M (molar), R represents the gas constant (1.988 cal/mol K), T represents the temperature (K) and [Activity] can be K_d, IC_{50} or EC_{50}.

3. Results and discussion

3.1. Characteristic bridge water and MM-PBSA binding energies

The inhibitory activity of IC_{50}, representing affinities of the wild type and Y181C HIV-1 RT complexes with nevirapine, was investigated through kinetic studies. Since the IC_{50} was converted to the experimental binding energies, as reported in the literature, a list of nevirapine activities against wild type and Y181C HIV-1 RT is shown in Table 1. The major mutation at position 181 from tyrosine to cysteine clearly caused nevirapine to lose its efficiency, as indicated by the higher concentration of nevirapine usage. Also, variations of binding affinities depended on the bioactivity assay of each laboratory. The conversion of binding affinities into energies is the link from the experimental to the computational approach. Experimental binding energies are also used as a key to compare to the MM-PBSA calculations for each model in this work.

The MM-PBSA calculations were acquired by sampling 500 snapshots from the trajectory. In classical MM-PBSA calculations all solvent and ions are normally removed. Therefore, the continuum solvent model was used instead; but this failed to reproduce the experimental binding energies in the HIV-1 RT/nevirapine system, as the binding energies of −32.20 and −28.07 kcal/mol found in wild type and Y181C RT complexes, respectively. Among the various complex structures of HIV-1 RT/nevirapine available, the structure code 1VRT is the highest resolution from X-ray crystallographic structure. Plenty of crystal water is found in 1VRT, including bridge water which is only present in this complex structure. Since crystallographic water molecules in the complex code 1VRT.pdb were considered as an important part, it had been integrated in the binding pocket throughout the simulations. It was found that WAT1067, located close to oxygen atom of nevirapine.

The characteristics of WAT1067 were analyzed from the trajectory. We found that WAT1067 forms a moderate/weak hydrogen-bonded bridge between nevirapine and the binding pocket of both wild type and Y181C HIV-1 RT, as shown in Fig. 1. WAT1067 acts as a hydrogen donor to O1(nevirapine), O(Leu234) and O(His235). In addition, this water molecule is also a hydrogen acceptor from N-H(His235). The heteroatomic distances between O(WAT1067) and O1(nevirapine), O(Leu234), O(His235) and N(His235) were measured within the last 2 ns of the trajectories, as shown in Fig. 2. Average distances of 2.76, 3.60, 3.38 and 3.55 Å were found in the wild type complex, while average distances of

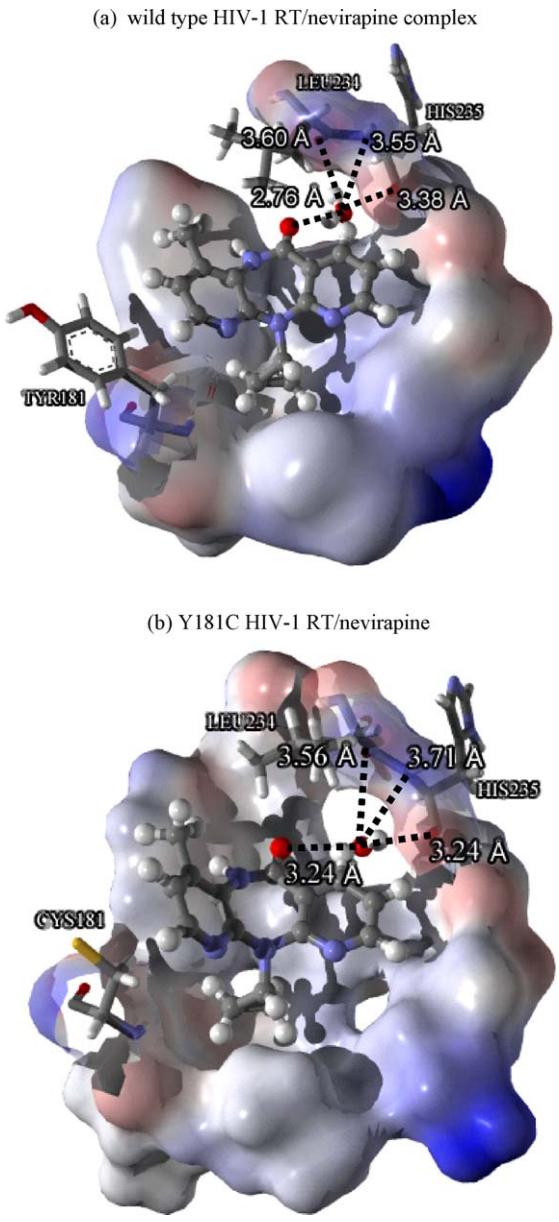

Fig. 1. Hydrogen bond network between the bridge water molecule, WAT1067, and residues in the binding pocket of HIV-1 RT present in the last snapshot of the simulations. Heteroatomic distances were measure in (a) wild type HIV-1 RT/nevirapine and (b) Y181C HIV-1 RT/nevirapine.

3.24, 3.56, 3.24 and 3.71 Å were found in the Y181C mutant type complex, corresponding to the distances between O(WAT1067) and O1(nevirapine), O(Leu234), O(His235) and N(His235), respectively. This suggests that the hydrogen bond formation between WAT1067 and nevirapine in the mutant RT complex was weaker than in the wild type RT. The space inside the binding pocket changed due to the Y181C mutation, allowing nevirapine and WAT1067 to move with more freedom. However, WAT1067 found new equilibrium and stability inside the pocket of Y181C RT/nevirapine, as shown in Fig. 3. A similar hydrogen bonded bridge position was also presented in the Monte Carlo simulations of the HIV-1 RT complex with nevirapine or MKC-442 [28]. Strong hydrogen bond interactions from the bridge water were also observed in another system, HIV-1 protease, as derived from quantum mechanical calculations [43]. Not only the bridge water, WAT1067, but three other water molecules – WAT1034, WAT1066 and WAT1183 – stay in contact with nevirapine at the beginning.

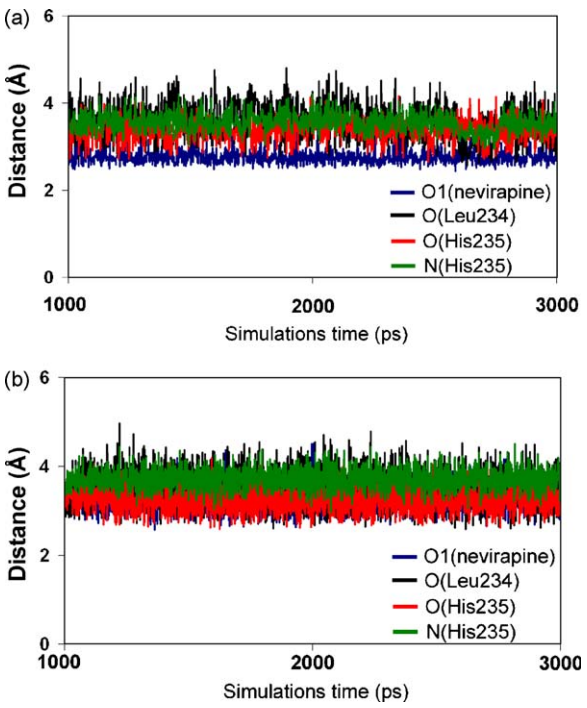

Fig. 2. Heteroatomic distances (Å) between WAT1067 and nevirapine, WAT1067 and selected residues, in the HIV-1 RT binding pocket were measured within the last 2 ns of the simulations. The distance between O(WAT1067) and O1(nevirapine) is represented in blue, O(WAT1067) and O(Leu234) in black, O(WAT1067) and O(His235) in red and O(WAT1067) and N(His235) in green. (a) Wild type HIV-1 RT/nevirapine and (b) Y181C HIV-1 RT/nevirapine.

During the whole RT complex simulations, only WAT1067 remained in both wild type and mutant type HIV-1 RT/nevirapine complexes. In the pocket of the wild type complex, WAT1034, WAT1066 and WAT1183 were absent. This is probably due to the hydrophobic properties of the RT pocket. On the other hand, the exchange of water found in the pocket of the Y181C RT complex when new water molecules, WAT1032, WAT1040 and WAT1057, came to substitute the former water position (see Figure 1 in supplementary data). Among the crystallographic structures of HIV-1 RT/nevirapine, there are eight structures that contain crystallographic water. However, bridge water is present only in 1VRT. The simulations suggest that water is able to move from place to place at all times. However, when comparing the water in the snapshot simulation structure with crystal structures (1JLF [50], 2HND [51], 2HNY [51], 1LWC [52], 1LWE [52], 1LWF [52], 1S1X [53] and 1VRT), the simulation waters showed good correspondence to the X-ray structure by locating in the solvent areas (see Fig. 4).

Due to the characteristics of the bridge water, WAT1067, as described above, the inclusion of only this water molecule changed the binding energies dramatically, as shown in Table 2. Shifts from −32.20 to −39.14 and −28.07 to −32.52 kcal/mol were found in the wild type and Y181C RT complexes, respectively. The major change of binding energy – from −32.20 to −39.14 kcal/mol in wild type HIV-1 RT/nevirapine, and from −28.07 to −32.53 kcal/mol in Y181C HIV-1 RT/nevirapine when WAT1067 was included in the MM-PBSA calculations – was evaluated by individual energy compositions. The main contribution comes from van der Waals interaction, whereas the bridge water significantly improves the electrostatic energy according to the binding process. This indicated that it was not only nevirapine and the RT residues but also one water molecule incorporated into the bound configuration that stabilized the complex (see Table 1 in supplementary data).

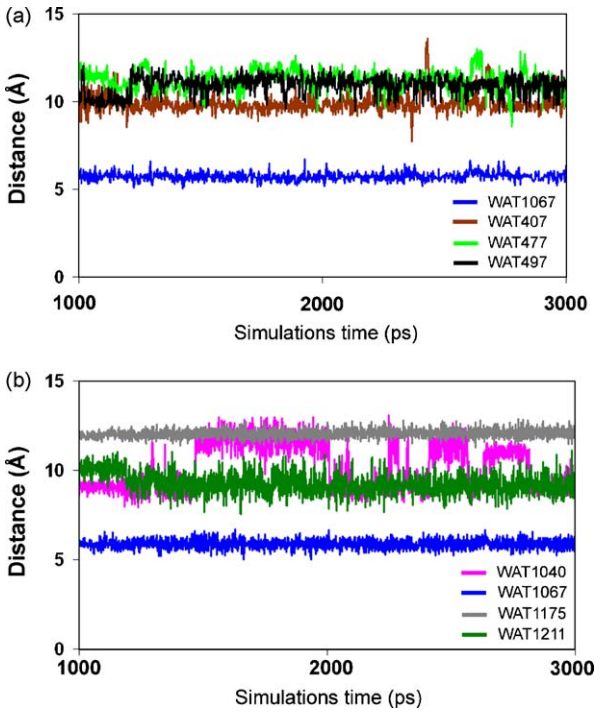

Fig. 3. Distances of the four closest water molecules to nevirapine measured by the center of mass (Å). (a) Wild type HIV-1 RT/nevirapine and (b) Y181C HIV-1 RT/nevirapine. Only WAT1067 presents in both the wild type and Y181C HIV-1 RT complexes (blue line).

While the appearance of WAT1067 proved to be a significant stabilizing force in the bonding between nevirapine and HIV-1 RT, especially in the mutant type complex, other water molecules were included to investigate their contribution as well. Each water molecule was added with respect to its distance to nevirapine. The distances, based on the center of mass from each water molecule to nevirapine in both the wild type and mutant type complexes, were investigated along with the simulations time. We found four water molecules closest to nevirapine: WAT1067, WAT407, WAT477 and WAT497 in the wild type RT/nevirapine; and WAT1040, WAT1067, WAT1175 and WAT1211 in the Y181C complex. Water identification numbers higher than 1001 represented the crystal water from the starting crystallographic structure, while the others (WAT407, WAT477 and WAT497) were solvated water that had been generated by the leap subprogram in AMBER during the setup process. Average distances of these four water molecules to nevirapine, calculated over the last 2 ns trajectories, are listed in Table 3. The distances of each water molecule from nevirapine were ranked from the closest to the farthest. Water molecules in the wild type HIV-1 RT complex were ranked as WAT1067, WAT407, WAT497 and WAT477 with distances of 5.7, 9.9, 11.0 and 11.2 Å, respectively. On the other hand, water molecules in the Y181C HIV-1 RT complex were ranked as WAT1067, WAT1211, WAT1040 and WAT1175 with distances of 5.9, 9.1, 10.5 and 12.1 Å, respectively.

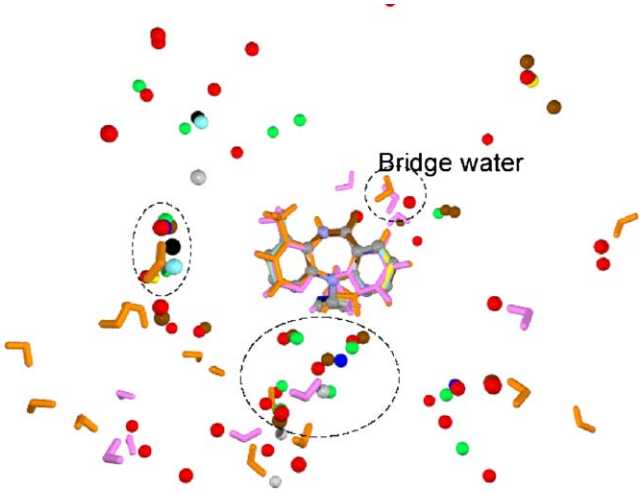

Fig. 4. Water accessible areas obtained by comparison of water position that surrounding nevirapine, while nevirapine in simulations (wild type HIV-1 RT (stick, pink) and Y181C HIV-1 RT (stick, orange)) and other crystal structures (2HND (brown), 2HNY (green), 1JLF (gray), 1LWC (blue), 1LWE (yellow), 1LWF (black) and 1S1X (light blue)) were superimposition referenced to nevirapine origin in structure of 1VRT (red).

Table 2
The MM-PBSA binding energies ($\Delta G^{MM\text{-}PBSA}$) of the wild type and Y181C HIV-1 RT/nevirapine complexes (kcal/mol).

	$\Delta G^{MM\text{-}PBSA}$ (kcal/mol)
1. Wild type HIV-1 RT + nevirapine	−32.20 (1.42)
2. Y181C HIV-1 RT + nevirapine	−28.07 (2.11)
3. Wild type HIV-1 RT + nevirapine + WAT1067	−39.14 (1.45)
4. Y181C HIV-1 RT + nevirapine + WAT1067	−32.52 (2.13)

Results obtained from classical MM-PBSA equation (i.e. model 1 and 2) and Eq. (2) (i.e. model 3 and 4).
Values in parenthesis show standard error in the calculations.

Table 3
Average distances of four water molecules closest to nevirapine measured by the center of mass from the wild type and Y181C HIV-1 RT complexes.

Wild type HIV-1 RT/nevirapine		Y181C HIV-1 RT/nevirapine	
WAT ID.	Average distance (Å)	WAT ID.	Average distance (Å)
1. WAT1067	5.7	1. WAT1067	5.9
2. WAT407	9.9	2. WAT1211	9.1
3. WAT497	11.0	3. WAT1040	10.5
4. WAT477	11.2	4. WAT1175	12.1

Distance fluctuation analysis of these four water molecules including the bridge water, as shown in Fig. 3, revealed the stabilities and deviations. Although all water molecules showed fluctuation, WAT1067 was the most stable throughout the simulations. Moreover, only WAT1067 was found to occupy the pocket without exchanges with other solvent molecules in both complexes (see Figure 1 in supplementary data). Even though the binding pocket was hydrophobic, one bridge water molecule was needed in the bound conformation. The thicker blue line in Fig. 3(b) shows that WAT1067 in the binding pocket of Y181C HIV-1 RT had a bit more freedom of space than in the pocket of wild type HIV-1 RT. Although this is a very minimal effect, it is a direct indication of the strength of hydrogen bond formation from this specific WAT1067. This stability of WAT1067 also corresponds to the previous hydrogen bonding distance analysis of the bridge water molecule. It was clear that the mutation at position 181 from tyrosine to cysteine affected the stability of the bridge water molecule. This is a new issue which explains the activity loss of nevirapine when the Y181C mutation occurs.

All four water molecules were considered in the MM-PBSA calculations, based on average distance analysis. However, a practical way to calculate the binding energies by including more than one water molecule could be done in two alternative ways. The calculated binding energies could be obtained either from the water molecule alone (following Eq. (1)) or kept with the enzyme receptor (following Eq. (2)). Energy calculations from each component based on the individual energy approach following Eq. (1) are listed in Table 4. In both cases of wild type and mutant

Table 4
MM-PBSA energies (kcal/mol) of the wild type and Y181C HIV-1 RT complexes with nevirapine calculated by including different numbers of solvent molecules based on individual energy.

Models	$\Delta G_{Complex}$	ΔG_{Enzyme}	ΔG_{Nev}	ΔG_{WAT}	$\Delta G^{MM\text{-}PBSA}$	Residual
RT^{WT}. + Nev. + 1WAT	−11467.39 (0.69)	−11283.98 (0.69)	−137.22 (0.04)	−7.05 (0.03)	−39.14 (1.45)	–
RT^{WT}. + Nev. + 2WAT	−11479.19 (0.69)	−11283.98 (0.69)	−137.22 (0.04)	−15.20 (0.02)	−42.79 (1.44)	−3.65
RT^{WT}. + Nev. + 3WAT	−11490.39 (0.72)	−11283.98 (0.69)	−137.22 (0.04)	−23.25 (0.02)	−45.94 (1.47)	−3.15
RT^{Y181C}. + Nev. + 1WAT	−11422.36 (1.04)	−11244.10 (1.02)	−138.69 (0.04)	−7.05 (0.02)	−32.52 (2.13)	–
RT^{Y181C}. + Nev. + 2WAT	−11440.62 (1.03)	−11244.10 (1.02)	−138.69 (0.04)	−15.13 (0.02)	−42.70 (2.11)	−10.18
RT^{Y181C}. + Nev. + 3WAT	−11454.34 (1.09)	−11244.10 (1.02)	−138.69 (0.04)	−23.09 (0.02)	−48.46 (2.17)	−5.76

RT^{WT}. = wild type HIV-1 RT.
RT^{Y181C} = Y181C HIV-1 RT.
Nev. = nevirapine.
RT^{WT} 1WAT = WAT1067.
RT^{WT} 2WAT = WAT1067 + WAT407.
RT^{WT} 3WAT = WAT1067 + WAT407 + WAT497.
RT^{Y181C} 1WAT = WAT1067.
RT^{Y181C} 2WAT = WAT1067 + WAT1211.
RT^{Y181C} 3WAT = WAT1067 + WAT1211 + WAT1040.
Values in parenthesis show standard error in the calculations.

type complexes, the obtained binding energies became lower after each addition of a water molecule. The difference of binding energies after each addition was reported as the residual number to evaluate the methodology. In the wild type RT complex, residuals of −3.65 and −3.15 kcal/mol were obtained after addition of the second and third water molecules, respectively. In contrast, residuals of −10.18 and −5.76 kcal/mol were obtained, respectively, in the Y181C RT complex. The trend of slightly different binding energies in the wild type complex and fluctuating differences in the mutant complex suggested that the individual energy component approach used in the MM-PBSA calculations led to unstable binding energies. However, the binding energies from another calculation approach – by keeping the water molecule as part of the receptor – produced more reliable results, as shown in Table 5. Only the first addition – the important bridge water molecule – lowered the binding energy by a huge amount, because of its significant contribution to the electrostatic attraction (see Table 1 in supplementary data). Residuals of −0.06 and 0.00 kcal/mol were obtained after addition of the second and third water molecules, respectively, in the wild type complex. Likewise, residuals of −0.15 and −0.05 kcal/mol were obtained in the Y181C RT complex. The trend of small change in residuals – close to zero or not significantly different – suggested that the embedded water molecules provided good stability of the binding energies. Thus the appropriate methodology of including a water molecule into the receptor part would be used for the later binding energy calculations. All these results strongly confirmed that only the bridge water molecule was essential in the binding mechanism between nevirapine and HIV-1 RT.

The most appropriate MM-PBSA binding energies from the wild type and Y181C HIV-1 RT complexes with nevirapine were −37.65 and −29.82 kcal/mol, respectively. Hence the improvement of estimating the binding free energy can be done by inclusion of a bridge water molecule in the binding site. This results in a closer match to the experimental data, as compared to the results of Zhou and co-worker (Predict I) [27] who found −54.78 and −48.48 kcal/mol for the wild type and Y181C RT/nevirapine, respectively, without including the bridge water molecule.

3.2. Fold resistance energies relative to the wild type HIV-1 RT

To determine the efficiency of nevirapine when the mutation occurred, the fold resistance value was calculated. The experimental data suggested that the fold resistance of nevirapine against Y181C HIV-1 RT could vary from about 50- to 500-fold, which was a rather wide range. The comparative results between experiment and modeling enabled the experimental fold resistance to be converted into the relative fold resistance energy, as shown in Table 6. The experimental relative fold resistance energies came from different laboratories, so the values varied from 2.35 to 3.64 kcal/mol. However, the previous computational prediction was 6.30 kcal/mol [27]. A similar number was found in this study when the MM-PBSA

Table 5
MM-PBSA energies (kcal/mol) of the wild type and Y181C HIV-1 RT complexes with nevirapine calculated by including different numbers of solvent molecules based on the embedded energy of WAT into the enzyme.

Models	$\Delta G_{Complex}$	$\Delta G_{Enzyme\text{-}WAT}$	ΔG_{Nev}	$\Delta G^{MM\text{-}PBSA}$	Residual
$RT^{WT\text{-}1WAT}$. + Nev.	−11467.39 (0.69)	−11292.52 (0.69)	−137.22 (0.04)	−37.65 (1.42)	–
$RT^{WT\text{-}2WAT}$. + Nev.	−11479.19 (0.69)	−11304.26 (0.69)	−137.22 (0.04)	−37.71 (1.42)	−0.06
$RT^{WT\text{-}3WAT}$. + Nev.	−11490.39 (0.72)	−11315.46 (0.72)	−137.22 (0.04)	−37.71 (1.48)	0.00
$RT^{Y181C\text{-}1WAT}$. + Nev.	−11422.36 (1.04)	−11253.85 (1.02)	−138.69 (0.04)	−29.82 (2.11)	–
$RT^{Y181C\text{-}2WAT}$. + Nev.	−11440.62 (1.03)	−11271.96 (1.01)	−138.69 (0.04)	−29.97 (2.08)	−0.15
$RT^{Y181C\text{-}3WAT}$. + Nev.	−11454.34 (1.09)	−11285.63 (1.07)	−138.69 (0.04)	−30.02 (2.20)	−0.05

RT^{WT}. = wild type HIV-1 RT.
RT^{Y181C} = Y181C HIV-1 RT.
Nev. = nevirapine.
$RT^{WT\text{-}1WAT}$ = wild type HIV-1 RT + WAT1067.
$RT^{WT\text{-}2WAT}$ = wild type HIV-1 RT + WAT1067 + WAT407.
$RT^{WT\text{-}3WAT}$ = wild type HIV-1 RT + WAT1067 + WAT407 + WAT497.
$RT^{Y181C\text{-}1WAT}$ = Y181C HIV-1 RT + WAT1067.
$RT^{Y181C\text{-}2WAT}$ = Y181C HIV-1 RT + WAT1067 + WAT1211.
$RT^{Y181C\text{-}3WAT}$ = Y181C HIV-1 RT + WAT1067 + WAT1211 + WAT1040.
Values in parenthesis show standard error in the calculations.

Table 6
Relative fold resistance energies ($\Delta\Delta G$) in kcal/mol for Y181C HIV-1 RT/nevirapine complex normalized to wild type HIV-1 RT/nevirapine complex.

	$\Delta\Delta G$ (kcal/mol)
Expt. [45] fold resistance = 468	3.64
Expt. [45] fold resistance = 131.6	2.89
Expt. [46] fold resistance = 53.3	2.35
Expt. [47] fold resistance = 312.5	3.40
Expt. [48] fold resistance = 69.1	2.51
Predict I [27]	6.30
(this work)	7.83

Fold resistance = $[\text{Activity}]_{\text{mutant}}/[\text{Activity}]_{\text{wild type}}$.
Relative fold resistance energy, $\Delta\Delta G_{\text{fold resistance}} = \Delta G_{\text{mutant}} - \Delta G_{\text{wild type}} = RT \ln(\text{Resistance Fold})$.
(this work) acquired data of free energy of mutant minus wild type from Table 5 (including one water).

calculations including the bridge water molecule gave a relative fold resistance energy of 7.83 kcal/mol.

3.3. Interaction energies

After the binding free energies and the relative fold resistance energies had been obtained, the interaction energies between nevirapine and binding pocket residues of HIV-1 RT were further investigated to break down the main contributions. The individual interaction energies between each RT residue and nevirapine were calculated. This decomposition energy calculation was based on an MM-GBSA approach. The decomposition energies were calculated on a pairwise per-residue basis, in which the interactions included the contributions from side chains and the backbone of the pocket residues. Then the interactions between each residue and nevirapine were calculated. Decomposition energies of the wild type and Y181C HIV-1 RT compared to other theoretical calculations are shown in Table 7. The decomposition showed that the main contributions were −4.30 and −5.74 kcal/mol from Leu100 and Tyr188, respectively, in the wild type RT complex. On the other hand, the main contributions of −4.24 and −4.94 kcal/mol were found from Leu100 and Tyr188, respectively, in the Y181C RT complex. Most of the decomposition energies for the wild type complex agree well with the interaction energies found from ONIOM (our own n-layered integrated molecular orbital and molecular mechanics) calculations [21]. Therefore, the decomposition revealed that the specific interactions changed from −3.12 to −2.44 kcal/mol at the position 181 mutation residue. This quantitative number explains clearly the loss of bound stability of nevirapine to the Y181C mutation in HIV-1 RT. Only a few interactions showed agreement between the decomposition and the MFCC (molecular fractionation with conjugate caps) approach [44]. Although the decomposition revealed that all of the interactions in the binding pocket were attractive forces, some repulsive energies were found in the ONIOM and the MFCC approaches. The repulsive forces were found at Leu100, Val179, Tyr181Cys, Gly190 and Leu234. These contradictory interactions at some amino acids occurred due to the geometry which had been used in the calculations. While the ONIOM and the MFCC approaches used a starting structure based on the crystallographic structure from the protein databank, the set of structures used in the decomposition energy calculations were taken by sampling from the MD trajectory. This introduced new information on how the dynamics of the binding pocket of HIV-1 RT – especially Leu100, Val179, Tyr181Cys, Gly190 and Leu234 – contributed to the nevirapine binding.

We further investigated the interaction between nevirapine and amino acids related to the bridge water molecule, since our analysis of MM-PBSA binding energies had proven the hydrogen bond bridge water molecule to be involved in nevirapine binding. The decomposition calculations via this bridge water molecule were investigated. The calculation of the decomposition energies from the bridge water molecule to nevirapine and the pocket residues, which refer to the hydrogen bond formation residues, are shown in Table 8. While the bridge water molecule formed similar levels of interaction to Leu234 and His235, significantly different interaction was found from nevirapine in the wild type and Y181C RT complexes (−2.36 and −0.56 kcal/mol, respectively). The lower attractive energy between WAT1067 and nevirapine in the mutant enzyme complex corresponded to the hydrogen bond distance analysis earlier. The larger freedom of WAT1067 in the Y181C RT complex caused the flexible distance between O(WAT1067) and O1(nevirapine); therefore, the attraction was broken. Losing interaction with the bridge water molecule had the important effect of making the nevirapine unstable inside the Y181C HIV-1 RT.

Moreover, if the decomposition energies via WAT1067 were taken into account, the attractive energies between Leu234, His235 and nevirapine (as shown in Table 7) had changed dramatically. Without the bridge water, nevirapine has attractive interactions of −3.03 and −1.19 kcal/mol to Leu234 and His235, respectively, in the wild type complex; and interactions of −2.43 and −1.15 kcal/mol to Leu234 and His235, respectively, in the mutant enzyme complex. When the attractive energies via the

Table 7
Interaction energies (kcal/mol) between nevirapine and the binding pocket residues of the wild type and Y181C HIV-1 RT.

Residues	Decomposition[a]		ONIOM[b]	MFCC approach[c]	
	Wild type	Y181C	Wild type	Wild type	Y181C
Pro95	−1.00 (0.10)	−1.29 (0.20)	−0.87	–	–
Leu100	−4.30 (0.43)	−4.24 (0.40)	−3.09	−0.58	2.81
Lys101	−0.71 (0.24)	−0.50 (0.17)	−1.10	−2.28	−3.08
Lys102	−0.26 (0.09)	−0.15 (0.06)	−0.37	−1.34	−1.25
Lys103	−1.92 (0.31)	−2.27 (0.40)	−1.20	−2.52	−2.38
Lys104	−0.09 (0.02)	−0.10 (0.02)	0.02	–	–
Ser105	−0.11 (0.03)	−0.08 (0.02)	−0.16	–	–
Val106	−2.99 (0.46)	−2.29 (0.30)	−0.08	–	–
Val179	−1.64 (0.26)	−1.89 (0.44)	1.47	–	–
Ile180	−0.72 (0.14)	−0.94 (0.11)	−0.50	–	–
Tyr181Cys	−3.12 (0.51)	−2.44 (0.34)	−2.79	1.34	7.63
Tyr188	−5.74 (0.40)	−4.94 (0.56)	−5.05	−2.07	−1.35
Val189	−1.11 (0.21)	−0.59 (0.13)	−0.64	–	–
Gly190	−0.84 (0.24)	−0.91 (0.13)	1.65	–	–
Phe227	−0.67 (0.27)	−0.72 (0.22)	−1.74	−2.23	−1.46
Leu228	−0.01 (0.00)	−0.02 (0.00)	−0.20	–	–
Trp229	−2.02 (0.30)	−1.52 (0.38)	−1.19	–	–
Leu234	−3.03 (0.40)	−2.43 (0.37)	1.22	–	–
His235	−1.19 (0.15)	−1.15 (0.20)	−2.39	−1.89	−0.64
Pro236	−1.30 (0.11)	−1.17 (0.17)	−1.63	−5.28	−2.41
Tyr318	−2.09 (0.40)	−2.17 (0.27)	−1.80	–	–
Glu138[d]	−0.21 (0.08)	−0.12 (0.04)	−0.09	–	–

Values in parenthesis show standard error in the calculations.
[a] Decomposition energies on a pairwise per-residue basis.
[b] Interaction energies calculated at the MP2/6-31G(d,p) level with BSSE corrected from ONIOM3 (MP2/6-31G(d,p):B3LYP/6-31G(d,p):PM3) optimization [21].
[c] Molecular fraction with conjugate caps approach [44].
[d] Glu138 taken from p51 domain of RT.

Table 8
Decomposition energies (kcal/mol) from the bridge water (WAT1067) to nevirapine and the key amino acids in the HIV-1 RT binding site.

	Wild type	Y181C
Leu234	−0.60 (0.39)	−0.69 (0.30)
His235	−1.74 (0.45)	−1.68 (0.55)
Nevirapine	−2.36 (0.45)	−0.56 (0.61)

Values in parenthesis show standard error in the calculations.

bridge water (Table 8) concerning hydrogen bond formation had been taken into account, the additional attractive energies of nevirapine to the bridge water, and the bridge water to Leu234 and His235, were included in the interactions between nevirapine and pocket residues (Leu234 and His235). Thus the total attractive energy of nevirapine and Leu234 changed from −3.03 to −5.99 kcal/mol for the wild type RT, and from −2.43 to −3.68 kcal/mol for the Y181C mutant type RT. The attractive energy of nevirapine and His235 changed from −1.19 to −5.29 kcal/mol for the wild type RT, and from −1.15 to −3.39 kcal/mol for the Y181C mutant type RT. These results suggested that if the attractive energies via the bridging water molecule were included, the interaction energies from Leu234 and His235 would become the major contributions, as high as that found from the Tyr188. These significant interactions indicated clearly how WAT1067 plays an important role in the binding pocket.

4. Conclusion

The combined calculation of MD/MM-PBSA plus the bridge water molecule is the key to investigating the wild type and Y181C HIV-1 RT/nevirapine complexes. The binding energy from additional water molecules showed that only the bridge solvent needed to be involved as a receptor part in the MM-PBSA calculations. This revealed the significant contribution from bridge water in the binding of HIV-1 RT and nevirapine. The relative fold resistance energies are largely greater than predicted (i.e., experiment shows 2.5–3.64 kcal/mol, and theory shows 6.30–7.83 kcal/mol). The bridge water formed hydrogen bonding to nevirapine on one side, and amino acids inside the binding pocket on the other side. WAT1067 acts as a hydrogen donor to O1(nevirapine), O(Leu234) and O(His235), as well as a hydrogen acceptor from N-H(His235).

Interaction energies between nevirapine and residues in the binding pocket of HIV-1 RT were obtained from the decomposition energies calculation. Nevirapine showed attractive energies to all enzyme residues within 7 Å in both wild type and Y181C RT complexes. While most of the interaction energies from decomposition analysis agreed with ONIOM, MFCC calculations, some contradictory spots were found because of the structural dynamics of the binding site pocket. Normally, the major contribution came from Tyr188 and Leu100 which have the strong interactions of −5.74 and −4.30 kcal/mol, respectively, for the wild type HIV-1 RT complex, and −4.94 and −4.24 kcal/mol, respectively, for the Y181C HIV-1 RT complex. But when the interactions via the bridge water were taken into account, the contribution from Leu234 and His235 became prominent as well, due to the interactions of −5.99 and −5.29 kcal/mol, respectively, for the wild type HIV-1 RT complex, and −3.68 and −3.39 kcal/mol, respectively, for the Y181C HIV-1 RT complex. The key interaction was that the attractive energies depended on the bridging water molecule. Apart from losing the interaction at position 181 when tyrosine was mutated to cysteine, nevirapine also lost interaction with the bridge water molecule. The lower stability of nevirapine inside the Y181C HIV-1 RT pocket was proven from the lack of attractive force. Therefore, nevirapine lacks potential against Y181C HIV-1 RT. The significant role of the bridging water molecule and its dynamic properties provide key information to strengthen the fundamental understanding of the binding of non-nucleoside HIV-1 RT inhibitors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2009.02.007.

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