The Molecular Basis of Resilience to the Effect of the Lys103Asn Mutation in Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors Studied by Targeted Molecular Dynamics Simulations

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Abstract: A series of targeted molecular dynamics simulations have been carried out in an attempt to assess the effect that the common Lys103Asn mutation in HIV-1 reverse transcriptase (RT) has on the binding of three representative non-nucleoside RT inhibitors (NNRTIs), nevirapine, efavirenz, and etravirine. We have shown previously that, in the absence of an incoming inhibitor, creation of the NNRTI binding pocket is hampered due to the existence of a hydrogen bond between the side chains of Asn103 and Tyr188 for which no equivalent exists in the wild-type enzyme. As an extension of this work, we now apply the same methodology to drive the enzyme’s conformation from the unbound state to the drug-bound state in the presence of the NNRTI. The location of each drug outside the binding pocket was determined by an automated docking program, and steering into the binding pocket followed a route that is likely to represent the actual entrance pathway. The additional hurdle to inhibitor entry imposed by the extra Asn103 hydrogen bond is seen to affect each NNRTI differently, with the ability to disrupt this interaction increasing in the order etravirine > efavirenz ≥ nevirapine, in good accord with the experimental findings. This coherent picture strongly suggests that attempts to overcome resistance through structure-based drug design may be considerably more successful if dynamic structural aspects of the type studied here are considered, particularly in cases where binding energy-based structure–activity relationship methods are unable to provide the required information.

Introduction

The Lys103Asn (K103N) escape mutation in HIV-1 reverse transcriptase (RT) is selected very frequently both in vitro and in vivo by numerous non-nucleoside RT inhibitors (NNRTIs) and is also commonly seen in patients receiving highly active antiretroviral therapy. The location of Lys103 (or Asn103) at the outer rim of the pocket where NNRTIs bind implies that it is very seldom involved in direct interactions with the bound drugs. This means that insight into the molecular basis of this particular resistance mutation is unlikely to be gained just from the static comparison of the complexes formed between the inhibitors and either the wild-type or the K103N mutant enzyme. For the same reason, the effect of this mutation is not amenable to structure–activity relationship studies that rely on differential interactions between a series of inhibitors and the whole or parts of the protein calculated for just one representative complex of each ligand. Nonetheless, important clues have been derived from several approaches including crystallographic and kinetic experiments and, more recently, theoretical calculations. Thus, early predictions of enhanced stabilization of the closed-pocket form of the K103N mutant RT in the unliganded state through hydrogen bonds that are not present in the wild-type enzyme were later confirmed when the crystal structure of the K103N apoenzyme (PDB code 1HQE) was solved. The proposed hydrogen bond between the side chains of Asn103 and Tyr188 was indeed observed and found to be additionally stabilized by interactions with two neighboring water molecules. Similar findings were reported for the complexes of the K103N mutant with HBY097 and l poviride, and also with efavirenz, PNU142721, and MSC194, all of which consistently showed that the K103N substitution induced only minor positional...

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approach was useful earlier to confirm the existence of a higher energy barrier to binding-site creation in the K103N mutant relative to the wild-type enzyme, but those simulations were carried out in the absence of an incoming inhibitor. In the following, we describe how we have probed possible entrance pathways into the binding site of wild-type and the K103N mutant RT for nevirapine, efavirenz, and etravirine, as representatives of first- and second-generation NNRTIs for which at least one crystal structure has been determined in complex with one of these RT enzymes.

Materials and Methods

A. Experimental Methods. a. Compounds. Nevirapine was from Boehringer Ingelheim, ddGTP was from Sigma Chemical Ltd. (St. Louis, MO), and etravirine was from Tibotec-Virgo (Belgium). Efavirenz was kindly provided by Dr. R. Kirsh and Dr. J.-P. Kleim (at that time at Hoechst AG, Frankfurt, Germany).

b. Site-Directed Mutagenesis of HIV-1 RT. The mutant RT-enzyme containing the Lys103Asn mutation was derived from the RT sequence cloned in pKRT2His. Site-directed mutagenesis was performed using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, Westburg, Leusden, The Netherlands) as described before. The two synthetic oligonucleotide primers (Invitrogen Life Technologies, Merelbeke, Belgium) used contained the mutation at amino acid position 103 of HIV-1 RT. The presence of the K103N mutation was confirmed by sequencing of the RT gene on an ABI Prism 3100 sequencer (Applied Biosystems, Foster City, CA) using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems).

c. Expression of Mutant Recombinant HIV-1 RT. Recombinant HIV-1 RT was expressed from a two-plasmid coexpression system as previously described. The p66 subunit of RT was expressed from pACYC66His and the p51 subunit from pKRT51. To construct wild-type and 103-mutated pACYC66His, wild-type and 103-mutated pKRT2His were digested with EcoRI and AvrII and the RT-containing fragments were ligated into pACYC184 digested with EcoRI and SalI. To construct wild-type and 103-mutated pKRT51, wild-type and 103-mutated pKRT2His were digested with Neol and Kpnl and the RT-containing fragment was ligated into pKRT51 digested with Neol and Kpnl. Expression and purification of recombinant RT was performed as described previously.

d. Reverse Transcriptase Assay. For determination of the 50% inhibitory concentration (IC50) of the test compounds against HIV-1 RT, the RNA-dependent DNA polymerase assay was performed as follows: the reaction mixture (50 µL) contained 50 mM Tris-HCl (pH 7.8), 5 mM DTT, 300 µM glutathione, 500 µM EDTA, 150 mM KCl, 5 mM MgCl2, 1.25 µg of bovine serum albumin, a fixed concentration of the labeled substrate [3H]dGTP (1.6 µM, 1 µCi; specific activity, 12.6 Ci/mmol; Amersham Pharmacia Biotech), a fixed concentration of the template/primer poly(rC):oligo(dG)12–18 (0.1 mM; Amersham Pharmacia Biotech), 0.06% Triton X-100, 5 µL of inhibitor solution (containing various concentrations [5-fold dilutions] of the compounds in the presence of a fixed concentration of 10% DMSO), and 5 µL of the RT preparations. The reaction mixtures were incubated at 37 °C for 30 min, at which time 200 µL of yeast RNA (2 mg/mL) and 1 mL of trichloroacetic acid (5%, v/v) in water were added. The solutions were kept on ice for at least 30 min, after which the acid-insoluble material was filtered over Whatman GF/C glass-fiber filters and washed 10 times with 2 mL of 5% trichloroacetic acid in water and one time with 70% ethanol. The filters were then analyzed for radioactivity in a liquid scintillation counter (Canberra Packard, Zellik, Belgium). The


Figure 1. Chemical structures of the non-nucleoside RT inhibitors studied.
IC_{50} for each test compound was determined as the compound concentration that inhibited HIV-1 RT activity by 50%.

B. Computational Methods. a. Quantum Mechanics Calculations. The geometries of nevirapine, etravirine, and efavirenz were optimized using the ab initio quantum chemistry program Gaussian 98 and the HF/3-21G* basis set. A set of atom-centered RHF 6-31G* basis function for each inhibitor was then obtained by using the RESP methodology, as implemented in the AMBER suite of programs (http://amber.sourceforge.net/). Covalent and nonbonded parameters for the inhibitor atoms were assigned, by analogy or through interpolation, from those already present in the AMBER force field (parm99), or consistently derived, as explained in more detail elsewhere.

b. Molecular Docking. Different conformers of nevirapine, etravirine, and efavirenz, generated by randomly changing torsion angles, were docked in different orientations near the putative entrance to the NNRTI binding site in HIV-1 RT using the genetic algorithm implemented in AutoDock. The wild-type unliganded (apo) enzyme form of RT (PDB code 1DL0) was used as the target protein. A volume for exploration was defined in the shape of a three-dimensional cubic grid (27 × 27 × 28 Å) with a spacing of 0.3 Å with the residues that are known to make up the NNRTI binding pocket in the p66 subunit and a long portion of the intersubunit cleft, including the surroundings of Glu138 in the p51 subunit. At each grid point, the receptor’s atomic affinity potentials for carbon, oxygen, nitrogen, sulfur, chlorine, fluorine, bromine, and hydrogen atoms were precalculated for rapid intra- and intermolecular energy evaluation of the docking solutions for each inhibitor. Despite the relatively wide variety of binding modes found by the automated docking program in the region delimited for exploration, most of the solutions were placed at the mouth of the putative entrance to the NNRTI binding pocket-to-be.

To obtain additional validation of the proposed binding mode for the more flexible etravirine, program GRID (http://www.moldiscovery.com/) was also used to search for sites on the enzyme that could be complementary to the functional groups present in this inhibitor.

For the GRID calculations, a 25 Å × 25 Å × 25 Å lattice of points spaced at 0.5 Å was established at the putative entrance close to the interface between the β7-β8 loop in p51 and the p66 subunit. The probes used were C(6) (aromatic carbon), N(6) (sp nitrogen with lone pair), and Br (bromine). The dielectric constants chosen were 4.0 for the macromolecule and 80.0 for the bulk water.

c. Molecular Dynamics Simulations. The macromolecular assemblies composed of the unliganded forms of heterodimeric wild-type (PDB code 1DL0) or K103N mutant (PDB code 1HQ6) HIV-1 RT and each inhibitor prealigned at the entrance of the binding cavity were solvated with a 20 Å-radius shell of TIP3P water molecules centered on Tyr188 of the p51 subunit. Each assembly was gradually refined in AMBER using a cutoff of 11.0 Å and a distance-dependent dielectric constant (ε = r_o) for dampening the electrostatic interactions. Given that the NNRTI binding site is close to the surface of the protein, and that only a “cap” of water molecules was used due to the large computational burden, a macroscopic dielectric constant is warranted as the full dampening effect of the solvent is not present.

First, only the water molecules were allowed to reorient, and then the water molecules and all protein side chains were allowed to relax. In each case, 500 steps of steepest descent were followed by conjugate gradient energy minimization until the root-mean-square (rms) value of the potential energy gradient was <0.01 kcal mol^{-1} Å^{-1}. The refined structures were then heated and equilibrated at 300 K for 100 ps prior to the tMD simulations at the same temperature during which the trajectories were biased so as to force pocket formation in the presence of the incoming inhibitor.

The structures used as end-points in the tMD simulations were: for nevirapine, the experimental complexes with either wild-type HIV-1 RT (PDB code 1VRT) or the K103N mutant enzyme (PDB code 1FKP); for efavirenz, the experimental complexes with wild-type and K103N HIV-1 RT (PDB codes 1IKW and 1IKV, respectively); and for etravirine, the X-ray complex with K103N RT (PDB code 1SV5). In the absence of an experimental structure, the same complex with Asn103 replaced with Lys. All of these target structures were solvated and adapted to the AMBER force field as reported above for the initial structures.

The tMD approach was essentially as described and made use of the standard implementation recently incorporated into AMBER (version 8.0), which allows the solvent molecules to move freely and follow the dynamics of the protein. A restraint was defined in terms of a mass-weighted rms superposition to the final reference structure (target) and applied in the force field as an extra energy term of the following form:

\[ E = 0.5k_B(N \text{rmsd} - \text{trmsd})^2 \]

where \( k_B \) is the force constant, \( N \) is the number of atoms, and trmsd is the target rms deviation, which we set to zero. A force constant of 0.5 kcal mol^{-1} Å^{-2} over 0.5 ns proved sufficient to find a low-energy path leading from the simulated structure to the target structure using only the heavy atoms of both protein and ligand in the rms definition. Note that this implementation is slightly different from that used previously for the enzyme alone in which the force constant was applied to all atoms.

Results and Discussion

General Considerations. In the process of cavity creation that accompanies NNRTI binding to the allosteric site in HIV-1 RT, the side chains of Tyr188 and Tyr181 reorient by “flicking” toward the polymerase active site. In the presence of the K103N mutation, however, formation of a good hydrogen bond between the phenol oxygen of Tyr188 and the side-chain carbamoyl of Asn103 was early suggested to hamper this rotation, thereby increasing the stabilization of the closed-pocket form of this mutant enzyme. Crystallographic evidence and results from tMD simulations have provided support to this hypothesis, which is in agreement with kinetic data, but to the best of our knowledge a comparative assessment of the binding of resilient and nonresilient NNRTIs to both the K103N mutant and the wild-type enzymes has not been reported.

In Vitro Activity of Several Representative HIV-1 Inhibitors. Etravirine (Figure 1) is an example of a second-generation NNRTI with a potency against wild-type HIV-1 RT and a wide range of HIV-1 RT mutants that appears to arise from its ability.
to adopt multiple conformations and orientations in the binding pocket.15 When we determined the concentration of this compound required to inhibit recombinant HIV-1 RT by 50% (IC\textsubscript{50}), virtually no differences were found between wild-type and K103N mutant enzymes (Table 1), in agreement with data reported by other authors.13 This was also the case for phosphonoforamic acid (PFA, foscarnet) and 2',3'-dideoxyguanosine-5'-triphosphate (ddGTP), which act through different mechanisms at the RT active site,32 and for capravirine, another second-generation NNRTI that is known to establish, at least with wild-type RT, an extensive network of hydrogen bonds involving main chain atoms of residues 101, 103, and 236 in the p66 subunit.33 On the contrary, the same mutation was found to increase the IC\textsubscript{50} of nevirapine and efavirenz by more than 20- and 35-fold, respectively, again in good accord with comparable data from the literature.10

### Table 1. Inhibitory Activity against Wild-type and Lys103Asn HIV-1 Reverse Transcriptases of Several Representative RT Inhibitors Belonging to Different Classes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>wild-type IC\textsubscript{50} (\textmu M)</th>
<th>K103N IC\textsubscript{50} (\textmu M)</th>
</tr>
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<tbody>
<tr>
<td>nevirapine</td>
<td>0.39 ± 0.085</td>
<td>&gt;10</td>
</tr>
<tr>
<td>efavirenz</td>
<td>0.004 ± 0.002</td>
<td>0.14 ± 0.064</td>
</tr>
<tr>
<td>etravirine</td>
<td>0.029 ± 0.014</td>
<td>0.032 ± 0.015</td>
</tr>
<tr>
<td>capravirine</td>
<td>0.005 ± 0.001</td>
<td>0.004 ± 0.001</td>
</tr>
<tr>
<td>TSAO-m^T</td>
<td>0.85 ± 0.085</td>
<td>8.5 ± 0.57</td>
</tr>
<tr>
<td>ddGTP</td>
<td>0.037 ± 0.002</td>
<td>0.016 ± 0.003</td>
</tr>
<tr>
<td>PFA (foscarnet)</td>
<td>5.4 ± 0.49</td>
<td>2.5 ± 0.74</td>
</tr>
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*50% inhibitory concentration or compound concentration required to inhibit recombinant HIV-1 RT by 50%. Template/primer: (poly)rC\textsubscript{30} -triphosphate (ddGTP), which act through different interaction energy maps: the pyrimidine amino group appeared hydrogen bonded to both the carboxylate group of Glu138 and the carbonyl group of Ile135 in the p51 subunit, and the 1,4-cyanophenyl moiety (ring II) was accommodated into a hydrophobic pocket lined by the hydrocarbon side chains of Leu100, Lys101, and Val179 in the p66 subunit.

Because the high flexibility of etravirine largely prevented any clustering, program GRID36 assisted in the selection of the preferred docking site. Thus, the solution chosen was that in which the functional groups of the molecules best matched the calculated interaction energy maps: the pyrimidine amino group appeared hydrogen bonded to both the carboxylate group of Glu138 and the carbonyl group of Ile135 in the p51 subunit, and the 1,4-cyanophenyl moiety (ring II) was accommodated into a hydrophobic pocket lined by the hydrocarbon side chains of Leu100, Lys101, and Val179 of p66.

Overall, this docking procedure provided us with a location for each drug that was taken as an unbiased and reasonable entry point for starting the subsequent MDS procedure. Remarkably, this location at the interface between the two subunits was reminiscent of the binding site proposed for the unique class of TSAO compounds,37 which are known to interfere with enzyme dimerization.39

**Targeted Molecular Dynamics Simulations.** The closed-pocket forms of the whole wild-type and K103N RT heterodimers (987 residues in all), as found in PDB entries 1DLO and 1HQE, but with the NNRTI bound at the putative entrance to the pocket as explained above, were gradually forced to adopt the conformation of their NNRTI-docked counterparts during an MD trajectory lasting 0.5 ns. In the initial apo-like conformation, the enzyme’s p66 thumb subdomain is folded down into the DNA binding cleft where it makes contacts with the tip of

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the fingers. Nonetheless, we must note that the structure of an unliganded HIV-1 RT that was produced by soaking out the weakly binding NNRTI, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT), from pregrown crystals revealed only minor rearrangements in this subdomain. At any rate, as the simulation progresses, the p66 thumb subdomain opens up and gradually extends away from the p66 fingers toward the target bound conformation (Supporting Information, Figure S1).

Concomitant with this, the $\beta_12-\beta_13-\beta_14$ sheet (the “primer grip”) is displaced so that the $\beta_12-\beta_13$ hairpin containing Phe227 and Trp229 (the back wall and floor, respectively, of the hydrophobic pocket where the inhibitors will eventually bind) is repositioned so that it now allows rotation of the side chains of Tyr181 and Tyr188 (Figure 5A–C). This is accompanied by the reported improper geometry of the catalytic aspartic acid residues 110, 185, and 186 at the polymerase active site that may result in enzyme inhibition, together with other proposed mechanisms such as hampering of some critical motions that are essential for the enzyme to perform catalysis and/or mispositioning of the nucleic acid template/primer substrate and the incoming dNTP.

The process of pocket creation in the presence of the NNRTI was similar to that studied in its absence, with one notable exception: when the side-chain conformations of Tyr181 and Tyr188 were monitored by measuring the evolution of their $\mathrm{N}-\mathrm{CA}-\mathrm{CB}-\mathrm{CG}$ dihedral angles, reorientation of Tyr188 was previously found to systematically precede that of Tyr181, whereas no such clear trend is observed in the present simulations (Figure 5). Because the inhibitors are now pushing their way into the binding pocket, the number and magnitude of the local barriers that have to be overcome to create the cavity and let the inhibitor in must be slightly different. In fact, the nature of these barriers appears to depend on the plasticity of the NNRTI as three different Y188 rotamers are observed in the simulation of etravirine with wild-type RT, whereas only two are detected in the simulations with the less flexible nevirapine and efavirenz. In any case, flipping of Tyr188 to adopt the pocket conformation in the K103N RT mutant enzyme is seen, in both sets of simulations, to be closely coupled to the breaking of the hydrogen bond between Asn103 and Tyr188 (Figure 5D). It is also apparent that this hydrogen bond is more easily broken when the NNRTI opening its way into the pocket-to-be is etravirine, in consonance with the resilience of this inhibitor to the effect of the K103N mutation (Table 1).

The progression of the conformational changes in the enzyme leading to creation of the NNRTI binding pocket, as well as the process of inhibitor entry into the cavity, were monitored by measuring the evolution of the rms deviation (rmsd) of both protein backbone atoms and inhibitor atoms along the simulation time (Figure 6). It can be clearly seen that, whereas the rmsd values decrease equally gradually and are virtually superimposable for the backbones of both proteins, the corresponding values for the inhibitors fall rather sharply and significant differences are apparent when the complexes of nevirapine and efavirenz with either the wild-type or the mutant enzyme are compared. The rmsd plateaus that are observed in the simulations of K103N HIV-1 RT with these two NNRTIs during the 300–400 ps period (Figure 6A,B) reflect the greater difficulty (longer times) in achieving the bound conformation in this mutant enzyme relative to wild-type. This delay is coincident with the longer time (and therefore higher energy) that is required to disrupt the hydrogen bond between Asn103 and Tyr188 in these two complexes when compared to the etravirine complex (Figure 5D). In contrast, the smooth drops in rmsd for etravirine in both complexes nicely overlap (Figure 6C), reflecting that the breaking of this hydrogen bond does not

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Drug outcompetes Tyr188 until, finally, both hydrogen bonds are lost in the docked complex corresponding to the X-ray crystal structure.\(^{15}\)

The feasibility of such NNRTI-RT interaction was assessed by searching for similar ones in other ligand–protein complexes present in the Protein Data Bank using the Relibase\(^{45}\) data retrieval system (http://relibase.ebi.ac.uk/). Several examples were indeed found, the most similar one being that taking place between the amide side chain of Asn768 in xanthine dehydrogenase and the cyano group present in inhibitor TEI-6720 (PDB accession code 1N5X). Interestingly, another NNRTI described in the literature, the phenylethylthiazolylthiourea (PETT) analogue MSC194, which also has a cyano substituent in a similar position, has nanomolar potency against wild-type RT that is reduced only 10-fold against the K103N mutant.\(^{10}\)

**Final Considerations.** The change at position 103 of HIV-1 RT from lysine (codons AAA and AAG) to asparagine (codons AAU and AAC) was rather intriguing for some time: despite being the most commonly observed NNRTI resistance mutation in the clinic, giving wide cross-resistance to most NNRTIs,\(^{46}\) this residue was only seldom seen to be involved in direct interactions with the inhibitors. The proposal that the resistance evoked by this mutation could be due to stabilization of the unliganded conformation of the enzyme by the formation of a hydrogen bond between the asparagine side chain and the hydroxyl group of Tyr188\(^9\) found experimental support when the crystal structure of the K103N RT enzyme was solved.\(^4\) Later, it was shown that creation of the cavity using a tMD approach required the use of higher force constants (or longer simulation times) to overcome the local energy barrier in the case of the K103N mutant RT than in the case of the wild-type enzyme.\(^{12}\)

At least in theory, the effect of stabilization of the unliganded form of the enzyme could be counterbalanced if a hydrogen bond could be formed between the asparagine side chain and a suitable moiety present at the right position in the incoming inhibitor. Despite the fact that up to now this has been mostly a trial-and-error exercise, principally achieved by focusing on the ligand already docked within the NNRTI binding pocket, some examples of success have already been published. A very significant one is capravirine,\(^{14}\) which shows no loss of potency for the Lys103→Asn mutation (Table 1), even though in the presence of this drug a threonine (encoded as ACA/ACC/ACG/ACU) rather than an asparagine is selected as an escape mutation.\(^{10}\) The change at position 103 of HIV-1 RT from lysine (codons AAA and AAG) to asparagine (codons AAU and AAC) was rather intriguing for some time: despite being the most commonly observed NNRTI resistance mutation in the clinic, giving wide cross-resistance to most NNRTIs,\(^{46}\) this residue was only seldom seen to be involved in direct interactions with the inhibitors. The proposal that the resistance evoked by this mutation could be due to stabilization of the unliganded conformation of the enzyme by the formation of a hydrogen bond between the asparagine side chain and the hydroxyl group of Tyr188\(^9\) found experimental support when the crystal structure of the K103N RT enzyme was solved.\(^4\) Later, it was shown that creation of the cavity using a tMD approach required the use of higher force constants (or longer simulation times) to overcome the local energy barrier in the case of the K103N mutant RT than in the case of the wild-type enzyme.\(^{12}\)

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References:
In this regard, it is of interest to analyze, in light of the results presented here, why resistance to efavirenz usually requires an additional mutation such as V108I or P225H besides that of K103N. Given the close proximity of the side chain of Val108 to the phenol ring of Tyr188, our simulations suggest that rotation of this ring during the process of pocket formation would be further hampered in the presence of the additional methyl group of an isolucine at this position. This hypothesis needs further exploration but may provide a satisfactory explanation to the observation that resistance is evoked despite the fact that most NNRTIs, including efavirenz, do not contact this residue in wild-type RT. In support of this claim is the observation that the greatest change observed in V108I RT is the mutation site. In the case of Pro225, which precedes the β12 strand (226–230), a mutation to His could affect the reported motions involving the β12-β13 hairpin, thereby also hampering pocket creation.

Insight into the flexibility of the different domains present in HIV-1 RT has been gained from visual comparison of many different crystal structures and also from application of a variety of computational methods including both the Gaussian network model of proteins and standard MD simulations. The present methodology represents a novel computational shortcut to study the binding of inhibitors to HIV-1 RT and can possibly be extended to other NNRTIs with the ability to bind in more than one conformation, such as the diaryltriazine (DATA) and diarylpyrimidine (DAPY) derivatives that led to rilpivirine, as well as to others that require the motion of only Tyr188 (e.g., HEPT) or neither Tyr181 nor Tyr188 (e.g., CP-94,707). In light of the useful information obtained herein for etravirine, and the clear distinctions established with respect to nevirapine and efavirenz, the hints provided by this type of simulations are likely to be of value in the design of novel resistance-evading inhibitors. Nevertheless, we are aware that more simulations, longer simulation times, and extensions to other inhibitors and mutant enzymes will be necessary to further validate this approach and expand our understanding of how to overcome this therapeutically important problem.

Conclusions

Lys103Asn is a clinically relevant mutation that hampers the binding of and confers resistance to many classes of structurally diverse NNRTIs inhibitors including efavirenz. Although it could be thought in principle that, in common with other frequent mutant RT enzymes, individual differences in efficacy between related NNRTIs could arise from differential interactions between the inhibitors and specific protein residues, crystallographic studies with the K103N mutant enzyme have revealed a notable absence of specific interactions involving the N103 side chain. On the other hand, evidence for enhanced stabilization of the closed-pocket form of the K103N mutant RT through Asn103–Tyr188 side-chain hydrogen bonding was obtained when the crystal structure of the K103N apoenzyme was solved. Support for this view was later gained when tMD simulations showed the existence of a higher energy barrier to pocket creation in this mutant enzyme relative to wild-type. The present results, in the presence of an incoming inhibitor, confirm previous findings, hint at plausible ways for NNRTI entry into the pocket, and further suggest that resilience to the insidious effects of the irksome K103N mutation can be attained by designing ligands that are able to disrupt the Asn103–Tyr188 interaction as they gain access into the binding site pocket rather than when they are already docked within the pocket, hence the necessity of studying these systems in a dynamic context. Furthermore, the intermediary protein structures that can be extracted from the tMD trajectories can provide alternative targets for ligand docking studies.

The development of etravirine and rilpivirine exemplifies the successful application, by a multidisciplinary team, of the concept of exploiting conformational degrees of freedom to offset the effects of resistance mutations. Implementation of the
methodology we now describe during the design process might help to devise alternative or complementary strategies to modify other lead compounds in such a way that they can circumvent the ability of RT enzymes harboring the common K103N substitution (and possibly other mutations) to escape the potency of the inhibitors. The method also opens new possibilities in the study of alternative entry pathways into the NNRTI binding pocket, as proposed, for example, for BHAP derivatives \(^{38}\) and CP-94,707.\(^{52}\)

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Supporting Information Available: Complete refs 5, 15, 16, 19, 28, 34, 41, 50, and 51, one additional figure showing the separate evolutions of the rmsd of the thumb domain and the \(\beta_{12}-\beta_{13}-\beta_{14}\) sheet along a representative simulation, and six Chime animations (html/xyz, one for each of the MD trajectories) that can be easily visualized in one’s favorite Internet navigator using the free Chemscape Chime plug-in (http://www.mdli.com/chemscape/chime/chime.html). This material is available free of charge via the Internet at http://pubs.acs.org.