

# Mutation Effects on 3D-Structural Reorganization Using HIV-1 Protease as a Case Study

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## Introduction

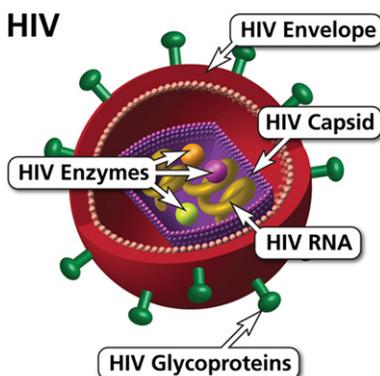
### AIDS and HIV

Acquired immunodeficiency syndrome (AIDS) spread by the human immunodeficiency virus (HIV) has become an epidemic worldwide (Sanou *et al.*, 2012). In 2016, it is assessed that about 36.7 million people were living with HIV, 19.5 million people were living with HIV on antiretroviral therapy, and a huge 1.8 million people were newly infected with HIV, and the infection is spreading at a shocking proportion. UNAIDS projections indicate that an additional 50 million people will be freshly infected in the coming decade, if the world doesn't get through to develop a potent therapy/medication (drugs or vaccine). However, remarkable progress against AIDS over the past 15 years has stimulated a global commitment to end the epidemic by 2030 (see "Relevant Websites section").

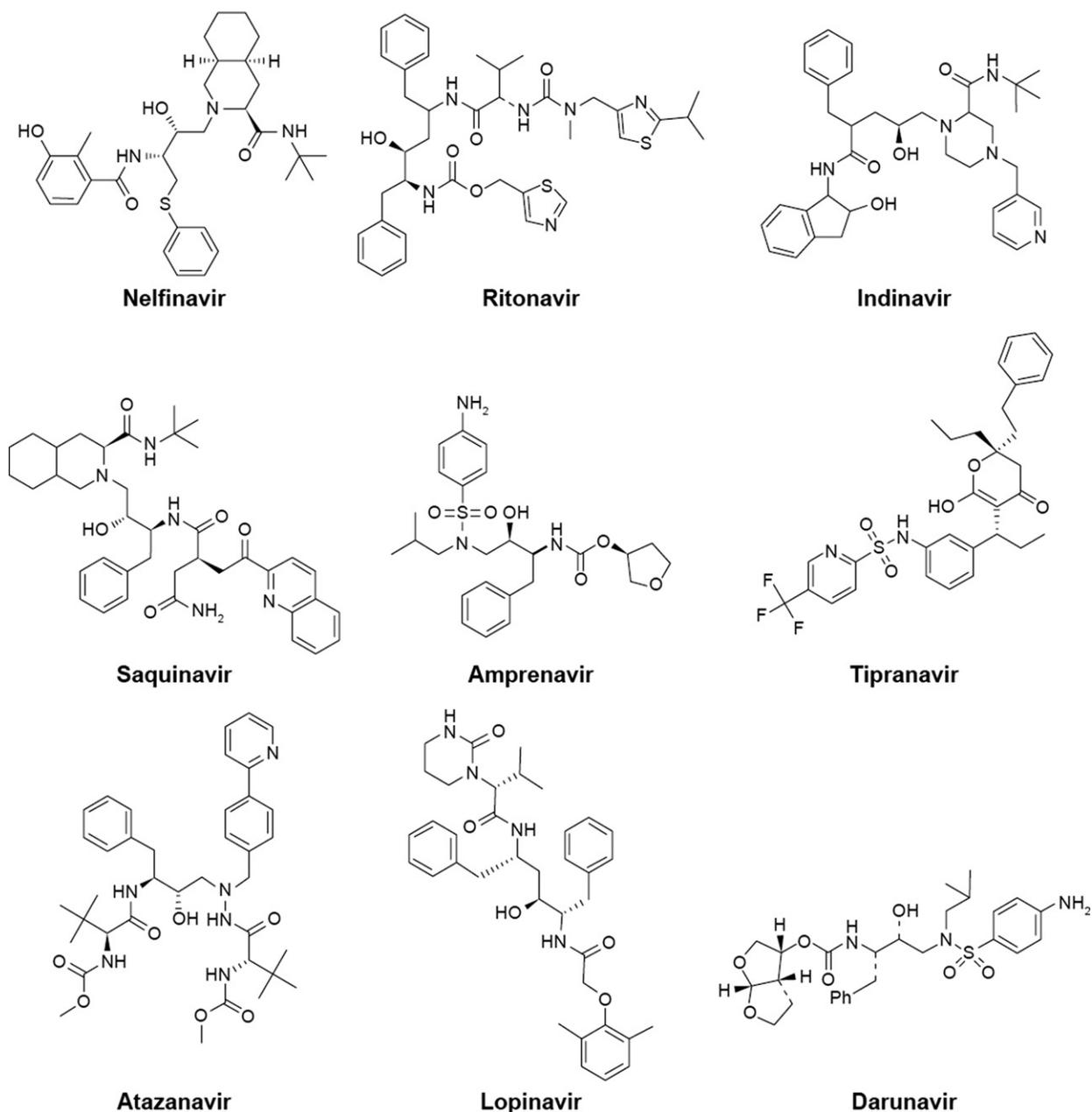
The lethal virus attacks the human immune system targeting the helper T-cells (specifically CD4<sup>+</sup> T cells), macrophages, and dendritic cells (Cunningham *et al.*, 2010) reducing the human immunity (Hatzioannou and Evans, 2012). Regardless of vigorous public health efforts and laborious research efforts, AIDS remains a fatal syndrome. Nevertheless, antiretroviral therapy has given a chance to tackle AIDS in part, but due to the clever HIV the goal for complete destruction of the epidemic remains distant. Mutation-induced drug resistance has abolished the clinical effectiveness of most of the FDA-approved drugs administered. So, there is a great demand for developing and designing a potent and less vulnerable drug for antiretroviral therapy. As shown in Fig. 1, HIV is a globular enveloped retrovirus, enclosing dual copies of single-stranded, positive-sense RNA (Ganser-Pomillos *et al.*, 2012). HIV infection starts with the attachment of the matured virus to the host cells containing CD4 + receptors and coreceptors CCR5 or CXCR4 with their envelope glycoproteins gp41 and gp120 (Tran *et al.*, 2012). Upon attachment, the host cell membrane and the viral envelope dissolves and the inner genomic content (RNAs) of the virus enters to the host cell. The RNA is then reversibly transcribes to viral DNA by the reverse transcriptase (Le Grice, 2012), which is followed by transcription, translation to form large polypeptide chain (Gag and Gag-pol). The large polypeptide chain is eventually cleaved by the proteolytic events by HIV-pr to form small structural and functional proteins of the virus. Subsequently, with the process of budding and assembly (Bukrinskaya, 2007), the synthesized proteins and part of host cellular membrane form the new virions for next phase of infections to the new CD4 + cells.

### HIV-1-Protease (HIV-Pr)

HIV-pr is an essential enzyme of HIV replication, and is a vital target for drug design strategies to fight AIDS. It cleaves the Gag and Gag-Pol polyproteins to generate the mature infectious virions capable of CD4 + cells infections (Fun *et al.*, 2012). Deactivating



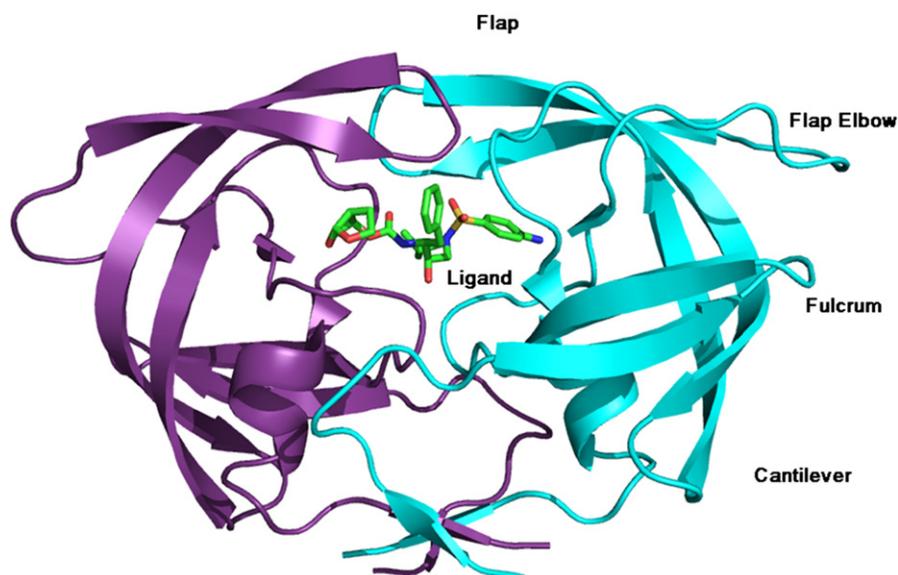
**Fig. 1** A schematic structure of a Human Immunodeficiency Virus type 1(HIV-1). Credit: National Institute of Health (NIH).



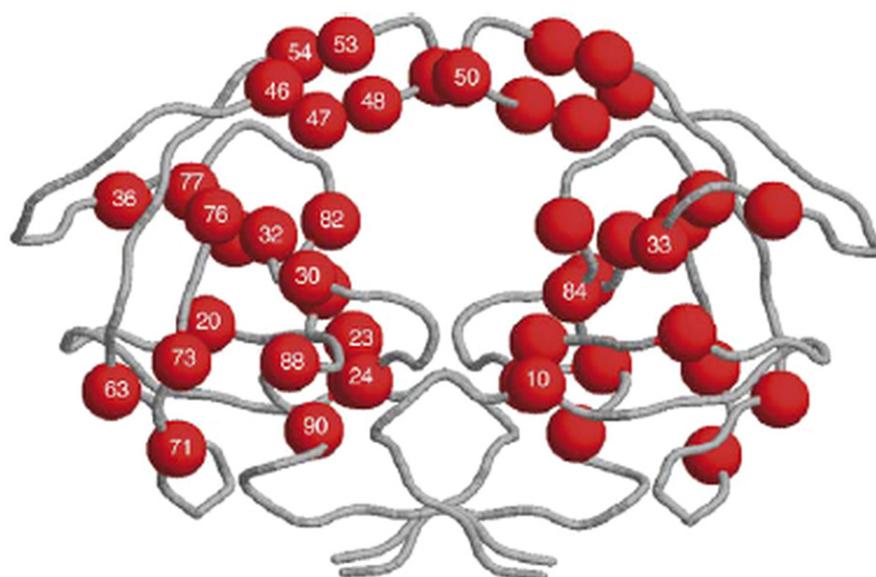
**Fig. 2** 2D structures of HIV-1 protease inhibitors that are approved by FDA to treat AIDS. Adapted from a research article Arodola, O.A., Soliman, M.E.S., 2015. "Could the FDA-approved anti-HIV PR inhibitors be promising anticancer agents? An answer from enhanced docking approach and molecular dynamics analyses." *Drug Design, Development and Therapy* 9, 6055–6065.

the enzyme's function creates immature virions without having the infectious supremacy. Keeping that in mind, several drugs has been designed, developed, and finally approved by FDA against AIDS. To date, at least nine FDA-approved protease inhibitors have been rolled out (**Fig. 2**), but none of them is effective after prolonged treatment time due to mutation-assisted drug resistance.

HIV-pr is a homodimeric aspartyl protease with C2 symmetric in the free form (**Brik and Wong 2003**), containing 99 amino acids in both of its chain-A and B. HIV-pr residues are numbered as 1–99 for chain A and 1'–99' for chain B. Flap (residues 43–58 and 43'–58'), flap elbow (residues 35–42 and 35'–42'), fulcrum (residues 11–22 and 11'–22'), cantilever (residues 59–75 and 59'–75'), and the active ligand binding site organize different regions of the enzyme (**Fig. 3**). The active site of the protein is formed by dimerization of the two monomers and is crowned by two identical flexible glycine rich flaps. The volume of the active site and size is controlled by dynamics of the flaps (**Piana et al., 2002a,b**). As a member of the aspartic protease family, the protease contains a catalytic triad (Asp25-Thr26-Gly27) in both the chains keeping functional aspartate residues at the dimer interface. The Asp residues are essential both catalytically and structurally while the Thr and Gly residues functions are still unknown at this time and are buried in the active site (**Mager, 2001**).



**Fig. 3** Schematic representation of the structure of HIV-1 protease (HIV-pr) bound to the ligand in the active site. Important regions of the protein are labeled. The homodimer protein is shown in violet and cyan ribbon structures.



**Fig. 4** The structural distribution of the most common mutations associated with drug resistance in the HIV-1 protease. Mutations can occur anywhere in the protease structure. Mutations within the binding cavity are very conservative and operate by distorting the shape of the cavity. Conformationally constrained inhibitors have difficulties in adapting to the altered geometry and lose significant binding affinity. Adapted from Overcoming HIV-1 resistance to protease inhibitors. *Drug Discovery Today. Disease Mechanisms | Infectious diseases*, vol. 3, No. 2, 2006.

### Mutations and Drug Resistance of HIV-Protease Inhibitors

Nevertheless, the HIV-pr-based therapeutic tactics have accomplished reasonable victory, but there are still some hurdles of serious side effects due to mutation-induced drug resistance. Currently, at least more than 50 mutations at near about 30 different codon positions of HIV-pr have been acknowledged. Lists of mutations that occur on the HIV-pr backbone are shown in [Fig. 4](#). A molecular level understanding of drug resistance requires the knowledge of both direct and indirect effects of mutation ([Johnson et al., 2010](#)). The mutant strains are increased in numbers by the drugs' abuse. Taking of numerous drugs has put selective pressure on the HIV leading to mutations and subsequent evolution of resistant variants ([Chen and Lee, 2006](#)). The mutations in HIV-pr are classified as those present near the active site (primary) and those appearing away from the active site (secondary). Both the

mutations affect the ligand/drug binding directly and indirectly by direct or indirect effects (Meher and Wang, 2012; Bandyopadhyay and Meher, 2006). There have been several studies (both experimental and computer simulation) indicating the importance of different mutations for the drug resistance in HIV-pr. Molecular dynamics (MD) simulation based approaches has been utilized by researchers worldwide to understand the HIV-pr 3D-structure dynamics and the drug resistance behavior (Piana *et al.*, 2002a, 2002b; Meher and Wang, 2012; Bandyopadhyay and Meher, 2006; Collins *et al.*, 1995; Hornak *et al.*, 2006; Meagher and Carlson, 2005; Ode *et al.*, 2006; Perryman *et al.*, 2004; Scott and Schiffer, 2000; Toth and Borics, 2006).

### HIV-pr 3D-Structure and its Dynamics

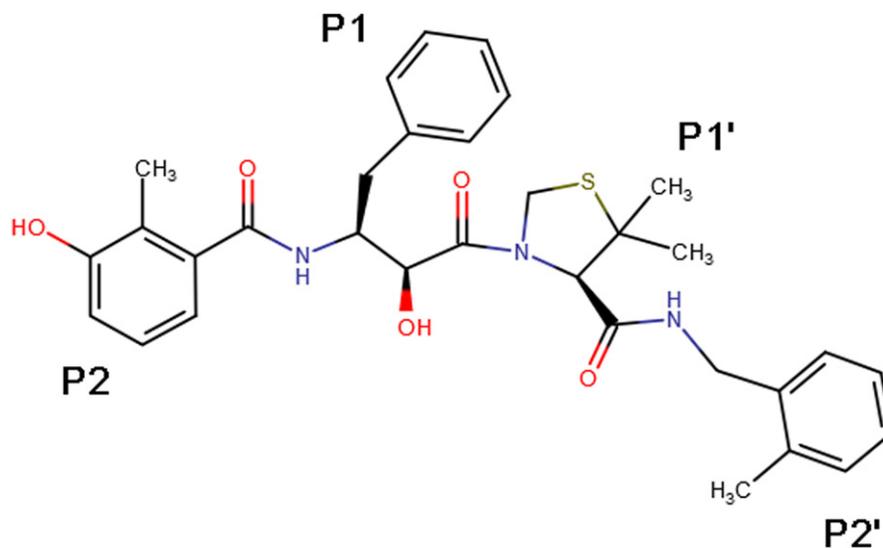
HIV-pr 3D-structural dynamics is mainly from the contribution of its flap and flap elbow dynamics, which have the maximum movements for accession of ligands or substrates in its active site region. To date, researchers have analyzed the dynamics of both unliganded and liganded forms of the HIV-pr. In all of the liganded forms, the flaps are pulled in toward the bottom of the active site, leading to a flap curling-in event. In the unliganded enzyme, flaps are shifted away from the active site (Hornak *et al.*, 2006) making the flaps curl out. The contribution of residues in the active site region and flaps to the stability is more distinct in the liganded form than in the unliganded form (Kurt *et al.*, 2003). However, in HIV-pr the anticorrelation movements of the flap-active site distances and fulcrum-flap elbow distances is more notable (Perryman *et al.*, 2004).

### Flap Movement and Dynamics

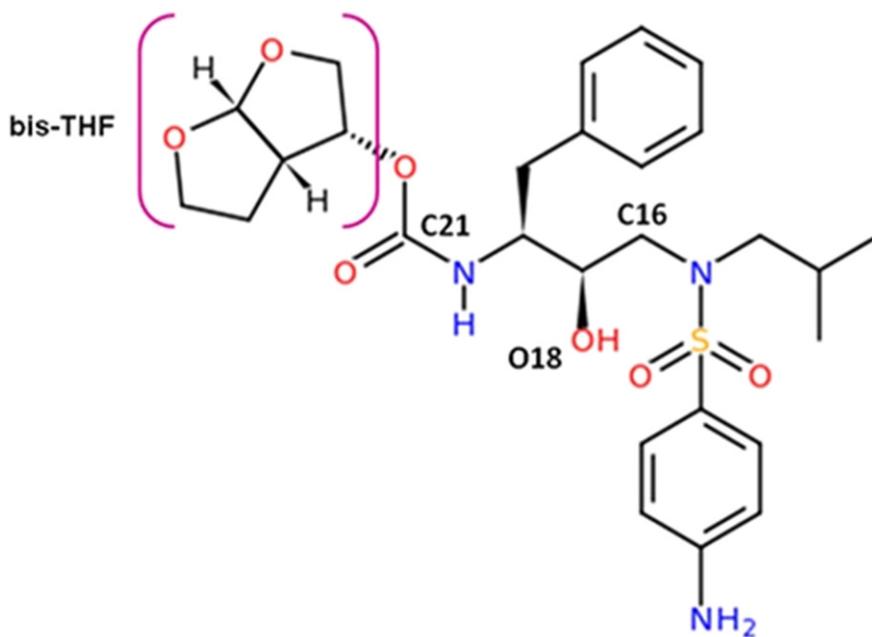
The flexibility of the flap tips (Gly48-Gly52 and Gly48'-Gly52') is known as flap dynamics. It opens and closes the flaps determining the cavity size. The conformational change in the flaps is correlated with structural reorganization of residues in the active site (Torbeev *et al.*, 2011). The mutations in flap region result in adjustment of the nonbonding interactions (van der Waals and electrostatic interactions) between the drugs and protein, subsequently helping drug resistance and rendering the drugs ineffective (Cai *et al.*, 2012). Thus numerous computational studies have made an effort to understand flap dynamics behavior. The effects of mutations on the 3D-conformational dynamics and reorganization of HIV-pr have been considered using MD simulations.

### Mutation Effects on HIV-pr 3D-Conformation

JE-2147 (Yoshimura *et al.*, 1999) is an experimental peptidomimetic HIV-pr inhibitor developed by Pfizer (Fig. 5). It was measured to be more effective than other prevailing HIV-pr inhibitors, which is potent against a wide range of HIV-1, HIV-2 strains. JE-2147 retains exclusive resistance profile (Kar and Knecht, 2012a) with two major mutations I84V and I47V. Nevertheless, I84V is common for other related ligands, I47V appears to be very particular for JE-2147 (Bandyopadhyay and Meher, 2006). The influence of I47V mutation is explored with MD simulation. Simulation outcomes showed greater flexibility of the side-chain of mutant Val47 than that of WT Ile47 in chain B of HIV-pr (Bandyopadhyay and Meher, 2006). Structural investigation exposed that the existence of a flexible P2' moiety is significant for the effectiveness of JE-2147 concerning wild-type (WT) and mutant viruses. These data propose that the use of flexible mechanisms may open a new opportunity for designing protease inhibitors with greater efficacy.



**Fig. 5** Molecular structure of the experimental inhibitor JE-2147. Four sites of interactions (P1, P2, P1', and P2') to the protein are labeled. Atoms are shown bonded to each other and are shown in solid lines. Atoms are shown in color as Carbon: Black, Oxygen: Red, Nitrogen: Blue and Sulfur: Gray.



**Fig. 6** Configuration of the inhibitor TMC114, with the moiety bis-THF enclosed in square bracket. Atoms playing critical role in the interactions between the inhibitor and the enzyme HIV-pr have been denoted in bold letters. Reprinted from Meher, B.R., Wang, Y., 2012. Interaction of I50V mutant and I50L/A71V double mutant HIV-protease with inhibitor TMC114 (darunavir): Molecular dynamics simulation and binding free energy studies. *Journal of Physical Chemistry B* 116, 1884–1900. With permission from J. Phys. Chem. B and publisher American Chemical Society (ACS).

TMC114, a nonpeptidic compound ended by the bis-tetrahydrofuran (bis-THF) moiety shown in Fig. 6, is an enormously effective protease inhibitor (PI) to deal with the drug resistant HIV strains. With the presence of the terminal bis-THF moiety, it slightly differs from its chemical analog, amprenavir. Several studies have shed light on the drug resistance behavior of HIV-pr mutants towards TMC114 (Meher and Wang, 2012, 2015; Kar and Knecht, 2012a, 2012b; Kovalevsky *et al.*, 2006b; Tie *et al.*, 2007; Chen *et al.*, 2010; Vaishnavi *et al.*, 2017).

#### **I47V mutation effect on JE-2147 and TMC114 binding**

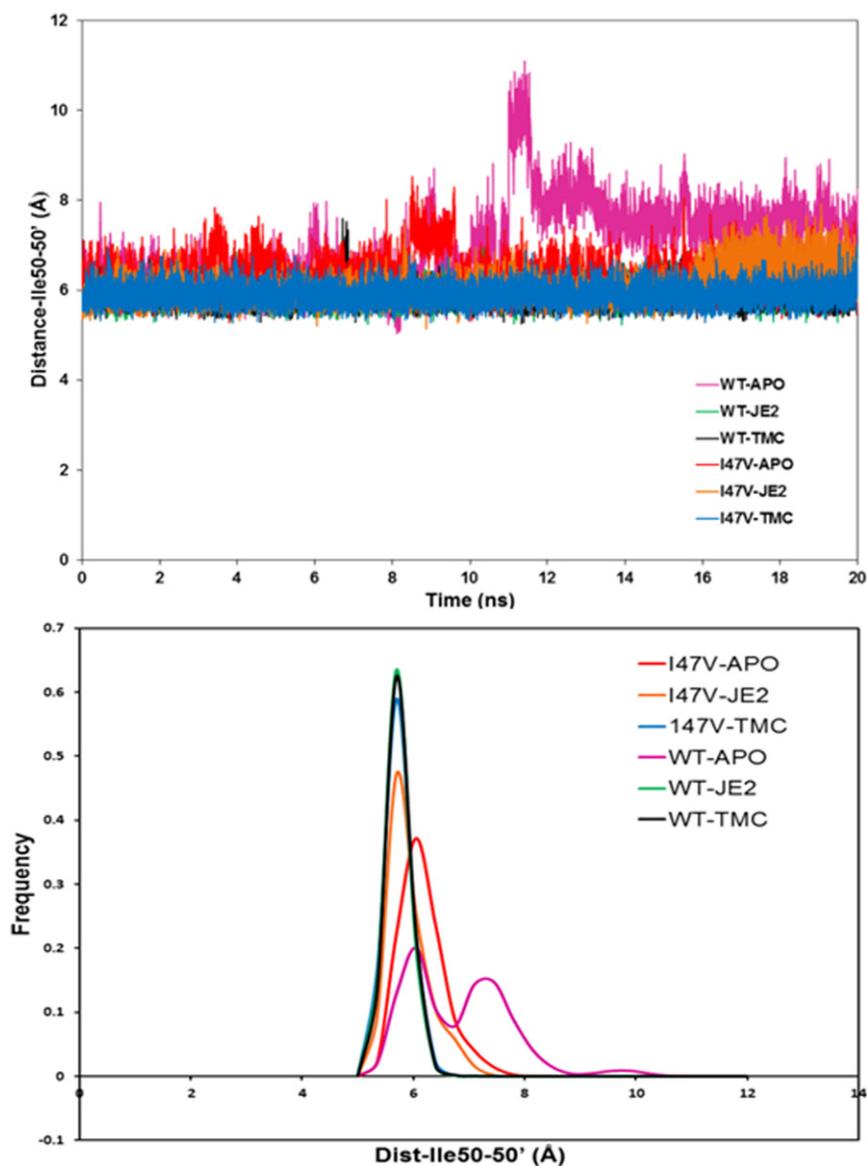
Vaishnavi *et al.* (2017) have investigated the binding of inhibitor TMC114 and JE-2147 to WT, and I47V mutant HIV-pr with all-atom MD simulations as well as MM-PBSA (molecular mechanics with Poisson–Boltzmann and surface area solvation) calculation. In I47V mutant apo HIV-pr, flap–flap distance was larger than WT or TMC114 and JE2 complexed mutant form (Fig. 7). The I47V-mutant complex HIV-pr has less curled flap tips and flexibility compared to WT and the apo mutant I47V. The mutant I47V decreases the binding affinity of I47V-HIV-pr to both the inhibitors (TMC114 and JE2), resulting in a drug resistance; due to an increased volume of the active site. (Fig. 9) However, the drug resistance of TMC114 to I47V mutant is heavier than JE-2147. The decrease of the binding affinity for the TMC114 complexed mutant I47V-HIV-pr is resultant of the the decreased electrostatic energy as well as van der Waals energy.

#### *Comparing the apo form of protein WT vs. Mutant*

The difference in RMSF (root mean square fluctuations) between the mutant and WT HIV-pr for each residue shows that the maximum changes in RMSF occurs between WT and mutant HIV-pr for the residues in the flap elbows of the two chains (35–42, 40′–42′), the dimerization region (Trp6), part of fulcrum (Gln18), and part of the cantilever region (67–69). MD simulation data from Vaishnavi *et al.* shows that the distance between the flap tip–active site and between flap tips has higher fluctuations for WT-APO and I47V-APO as expected. (Fig. 8).

#### *Comparing the complexed form of protein WT vs. Mutant*

In the complexed form HIV-pr, the difference in RMSF between WT and I47V-mutant is reduced for most of the residues. It was observed that regions around residues like Pro39-Trp42 (for TMC complex), and Trp6′–Arg8′ (for JE2 complex) shows remarkable fluctuations compared to WT with more than 0.75 Å. The relatively larger RMSF of the mutant I47V-complex to its apo-form counterpart is likely to be arising due to larger conformational fluctuations and weaker binding. The distance between Ile50–Ile50′ was determined to measure the relative motion of the flap tips. The distance variation between the complexed WT, I47V-TMC and I47V-JE2 HIV-pr was found to be fewer and tighter than apo HIV-pr (Fig. 6). Flap dynamics analysis also suggests larger active site volume in case of I47V-TMC and I47V-JE2. The results of these studies indicate that although the Ile50–Ile50′ distance was similar in

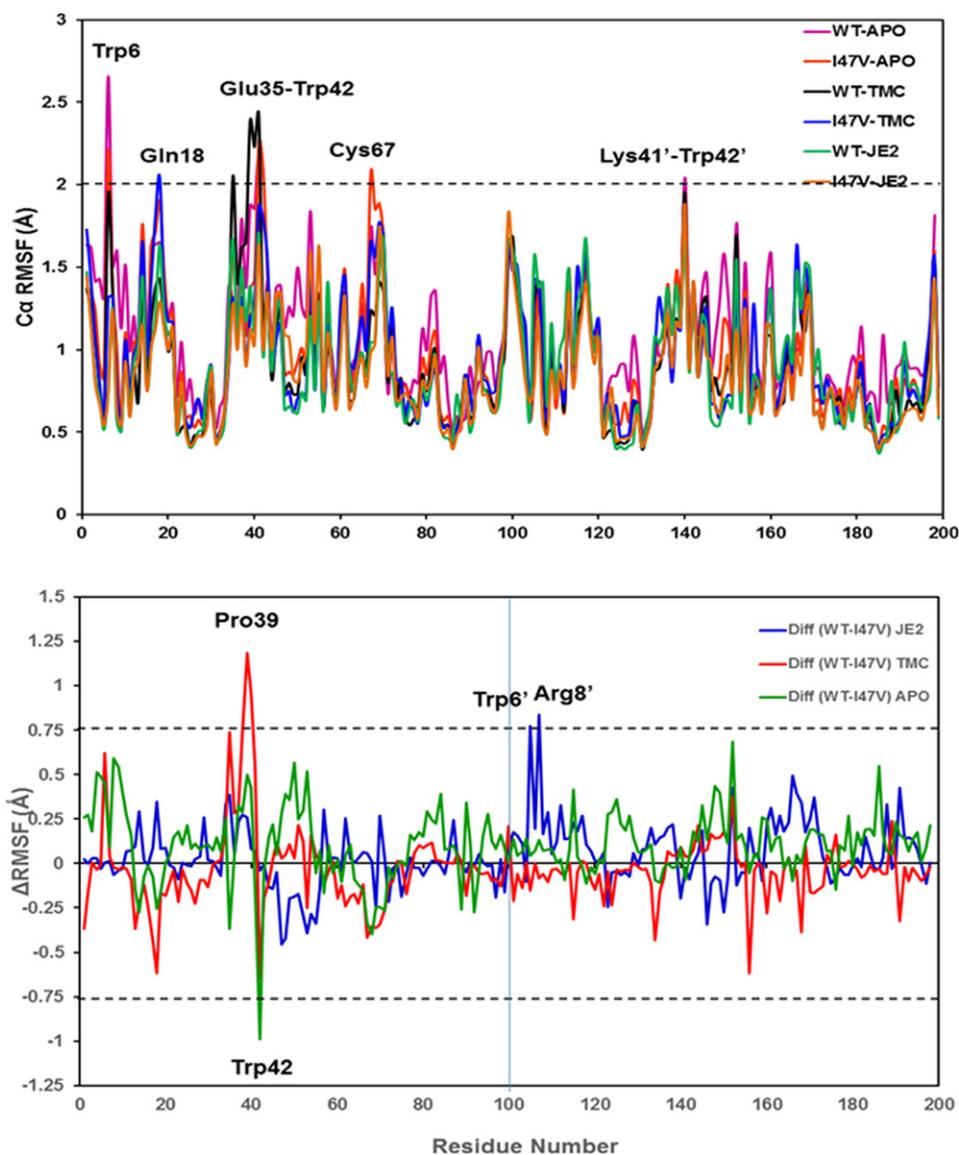


**Fig. 7** Time-series (above) and frequency distribution (below) plots for the flap tip–flap tip distances for the HIV-pr WT vs I47V mutant complexes and apo-type.

the complexed HIV-pr there still exist differences in the Asp25'-Ile50' distance, indicating the unique behavior of the two chains of a homodimeric enzyme like HIV-pr.

#### *Molecular mechanism of drug resistance*

In the I47V-mutant HIV-pr, the substitution of isoleucine with valine leads to the removal of a methyl group, which is likely to be decreasing the interaction with the central phenyl of TMC114 through C-H... $\pi$ . Also, it is likely to be shortening the hydrophobic side chain and increasing the size of the active site, resulting in a reduced binding affinity to TMC114 and JE-2147. This change results in a decrease of van der Waals energy between Val47 and TMC114 comparative to the WT. However, for Val47 (47') the change shows a significant decrease in van der Waals energy, which could possibly be due to the lessening of C-H...O interactions between the Val47 side chains and the P2' position of JE-2147 and Val47 side chains and bis-THF (bis-tetrahydrofuran) moiety. The calculated binding free energies of complexes WT-JE2, I47V-JE2, WT-TMC, and I47V-TMC are  $-31.03$ ,  $-29.76$ ,  $-34.43$ , and  $-30.73$  kcal/mol, respectively, indicating that the binding free energy of WT is higher than the mutant I47V. The binding affinities ( $\Delta G$ ) of I47V-JE2 and I47V-TMC complexes decrease by 1.27 and 3.70 kcal/mol from their WT counterpart, suggesting drug resistance for both the mutant. Residues like Val47' in I47V-JE2 complex directly lowers the  $\Delta G$  along with other residues like Gly49 and Val82' (Fig. 9).



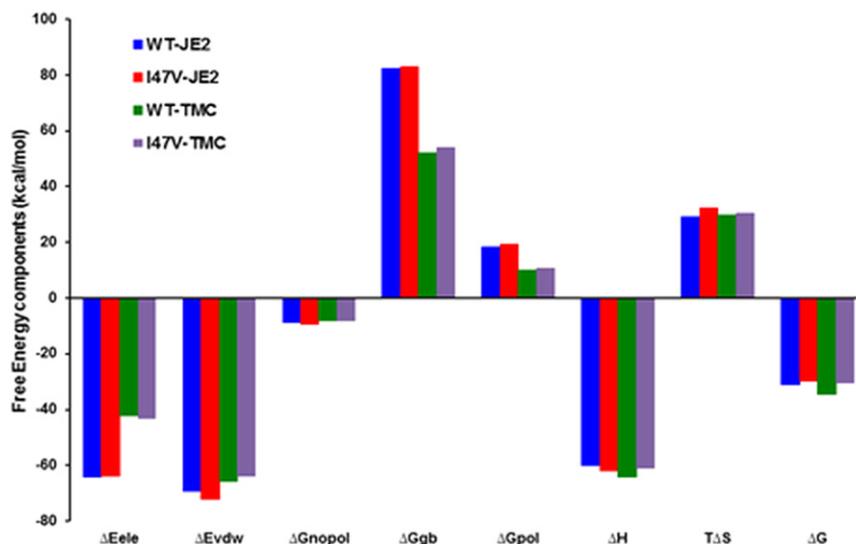
**Fig. 8** C $\alpha$  RMSF plot for the HIV-pr WT vs. I47V (above) and the difference is shown (below).

### ***I50V and I50L/A71V mutations effect on TMC114 binding***

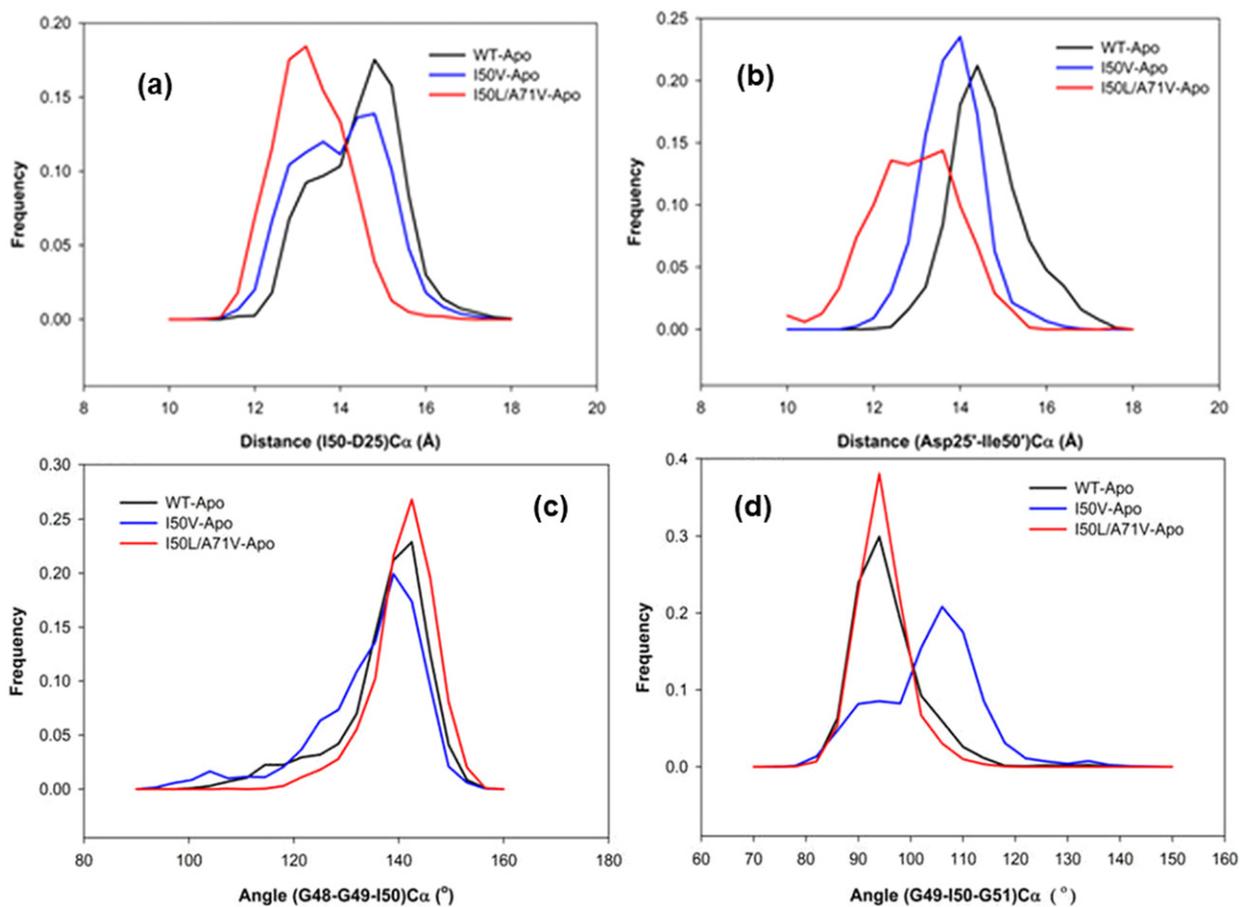
The single mutation I50V and the double mutation (I50L/A71V) are recognized as two important residue point mutations in HIV-pr which affect protease inhibitors efficacy. Though both the mutations are located at critical region of HIV-pr structure, and their effect on other protease inhibitors have been studied previously, the effect of I50L/A71V mutation on TMC114 binding and the mechanism for drug resistance is still elusive. Meher and Wang (2012) explored the binding of TMC114 (Fig. 6) to WT, single (I50V) along with the double mutant HIV-pr with all-atom MD simulations and MM-PBSA calculation. The analysis of the apo and complexed HIV-pr indicates that the flap curling and opening events in double mutant I50L/A71V are more stable than WT or I50V. Further, the flap-flap and flap-active site distances also appears to be smaller in I50L/A71V when compared to that of WT or I50V (Fig. 10), resulting in a compact active site with smaller volume. I50V mutant reduces the binding affinity to inhibitor TMC114, causing drug resistance; while the I50L/A71V double mutant escalates the binding affinity (Fig. 11). It is remarkable to observe that the I50L/A71V escalates the binding affinity possibly by the stronger binding and better adaptability of the inhibitor TMC114 in its active site.

### ***Comparing the apo form of protein WT vs. Mutant***

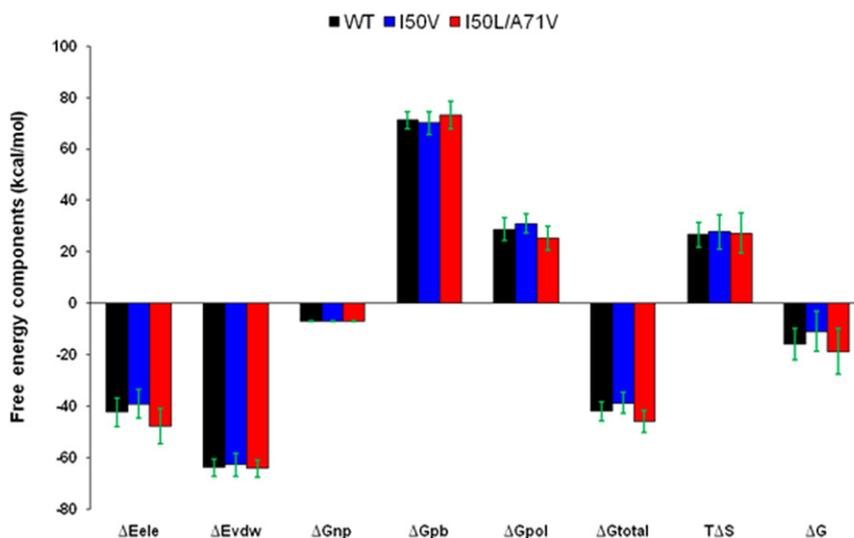
Difference in B-factors or isotropic temperature factors could offer direct perceptions into the structural variations of HIV-pr in its WT and mutant forms. Each residue difference in B-factors amongst the mutant and WT HIV-pr is highest in the dimer interface region (6, 8 and 6', 8'), flap elbow-A (35, 37, 39–41), and flap-A (49–52). Analysis of the WT and mutant simulations



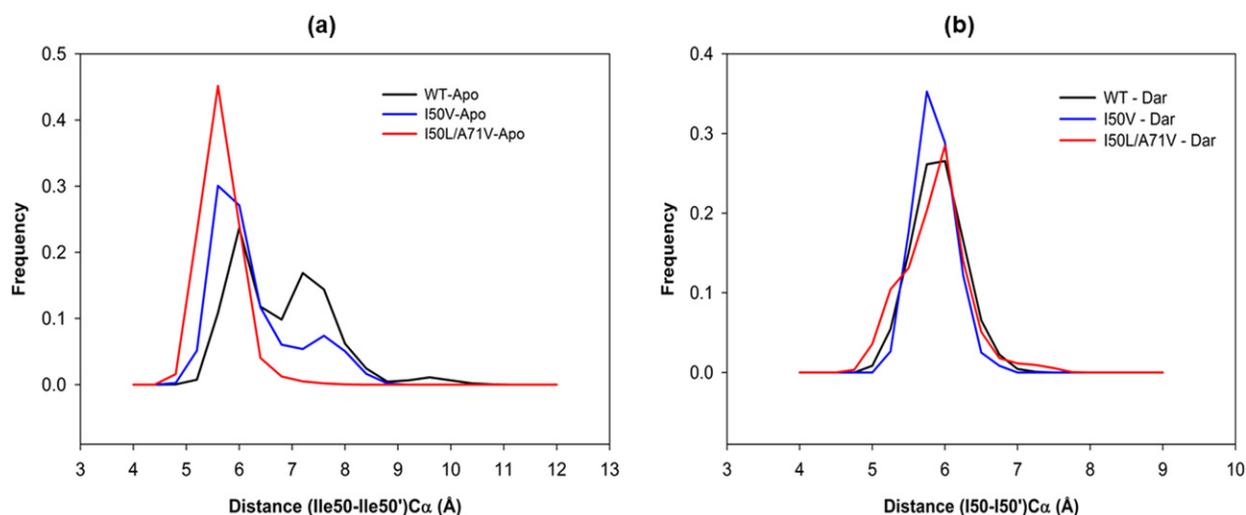
**Fig. 9** Energy components (kcal/mol) for the binding of TMC114 and JE-2147 to the WT and I47V mutant:  $\Delta E_{ele}$ : Electrostatic energy in the gas phase;  $\Delta E_{vdw}$ : Van der Waals energy;  $\Delta G_{nopol}$ : Nonpolar solvation energy;  $\Delta G_{gb}$ : Polar solvation energy;  $\Delta G_{pol}$ :  $\Delta E_{ele} + \Delta G_{gb}$ ;  $T\Delta S$ : Total entropy contribution;  $\Delta G_{total} = \Delta E_{ele} + \Delta E_{vdw} + \Delta E_{int} + \Delta G_{pb}$ ;  $\Delta G = \Delta G_{total} - T\Delta S$ .



**Fig. 10** Variability of histograms for the (a) Ile50–Asp25 distance; (b) Ile50' –Asp25' distance; (c) Gly48–Gly49–Ile50 TriCa angle; and (d) Gly49–Ile50–Gly51 TriCa angle for WT, I50V and I50L/A71V mutants' HIV-pr simulation of the apo-type. Reprinted from Meher, B.R., Wang, Y., 2012. Interaction of I50V mutant and I50L/A71V double mutant HIV-protease with inhibitor TMC114 (darunavir): Molecular dynamics simulation and binding free energy studies. *Journal of Physical Chemistry B* 116, 1884–1900. With permission from *J. Phys. Chem. B* and publisher American Chemical Society (ACS).



**Fig. 11** Energy components (kcal/mol) for the binding of TMC114 to the WT, I50V and I50L/A71V:  $\Delta E_{ele}$ : Electrostatic energy in the gas phase;  $\Delta E_{vdw}$ : Van der Waals energy;  $\Delta G_{np}$ : Nonpolar solvation energy;  $\Delta G_{pb}$ : Polar solvation energy;  $\Delta G_{pol}$ :  $\Delta E_{ele} + \Delta G_{pb}$ ;  $T\Delta S$ : Total entropy contribution;  $\Delta G_{total} = \Delta E_{ele} + \Delta E_{vdw} + \Delta E_{int} + \Delta G_{pb}$ ;  $\Delta G = \Delta G_{total} - T\Delta S$ . Error bars in green solid line indicates the difference. Reprinted from Meher, B.R., Wang, Y., 2012. Interaction of I50V mutant and I50L/A71V double mutant HIV-protease with inhibitor TMC114 (darunavir): Molecular dynamics simulation and binding free energy studies. Journal of Physical Chemistry B 116, 1884–1900. With permission from J. Phys. Chem. B and publisher American Chemical Society (ACS).

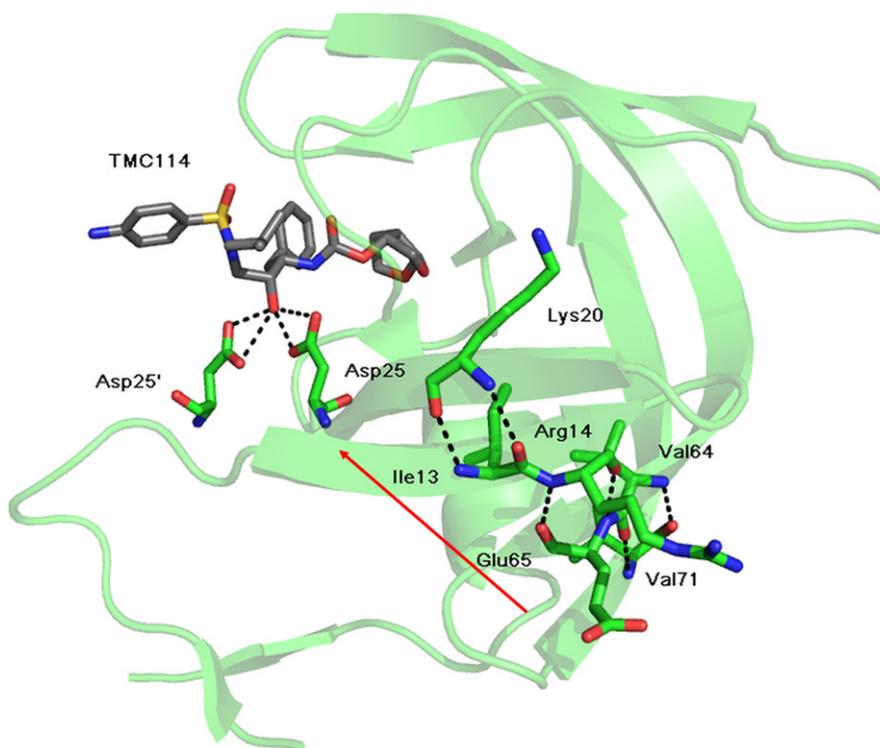


**Fig. 12** Histogram distribution of distance between the flap tips in the (a) apo form and (b) TMC114 complexed form for WT, I50V, and I50L/A71V HIV-pr. Reprinted from Meher, B.R., Wang, Y., 2012. Interaction of I50V mutant and I50L/A71V double mutant HIV-protease with inhibitor TMC114 (darunavir): Molecular dynamics simulation and binding free energy studies. Journal of Physical Chemistry B 116, 1884–1900. With permission from J. Phys. Chem. B and publisher American Chemical Society (ACS).

demonstrates that the flap–flap distance changes more in the WT and I50V than I50L/A71V (Fig. 12(a)), and flap–active site distance is measured to be smaller in I50L/A71V than in WT and I50V (Fig. 10). Therefore, both the flap–flap and flap–active site distance results recommend a neighboring movement of flaps in I50L/A71V comparing WT and I50V and possibly modifying the active site size reduced, which could help in improved binding of the TMC114 to the active site region. Improved binding of the TMC114 might be due to the increase in van der Waals (vdw) contacts between the TMC114 and the HIV-pr residues.

#### Comparing the complexed form of protein WT vs. Mutant

All the complexes (WT vs mutants) show similar fashion of dynamic features from the B-factor perspectives albeit a few exceptions. B-factor difference between the TMC114 complexed and apo HIV-pr for WT and mutant shows that the B-factor is reduced for most of the residues, specifically noticeable in the flap tip and flap elbow regions. It was observed that four regions around



**Fig. 13** Graphical representation of H-bond propagation in the double mutant I50L/A71V and Asp25(25')-TMC114 flip-flop interaction. Red color arrow shows the path of propagation.

17 (17'), 41 (41'), 53 (53'), and 70 (70') exert the highest dynamic fluctuations. The slightly reduced B-factor of the I50L/A71V-complex may be described by the comparatively less conformational variations and stronger binding. The reduced flexibility in the inhibitor-binding site directs to the reduction in  $K_m$ , resulting in an increase in the affinity of the enzymes for the inhibitor and stronger binding (Zoldak *et al.*, 2004). In order to understand the flap dynamics behavior, the Ile50-Ile50' distance was studied. The difference among the complexed WT, I50V, and I50L/A71V HIV-pr was observed to be fewer and thinner than that of the apo HIV-pr (Fig. 12(b)). The utmost distinct motion for the HIV-pr complex was found to be the side-chain mobility of catalytic Asp25 about the inhibitor TMC114. It shows a flip-flop interaction of the Asp25 OD1/OD2 atoms with the O18 of TMC114 may be originated by the change in H-bonding pattern of the double mutant induced from A71V mutation (Fig. 13).

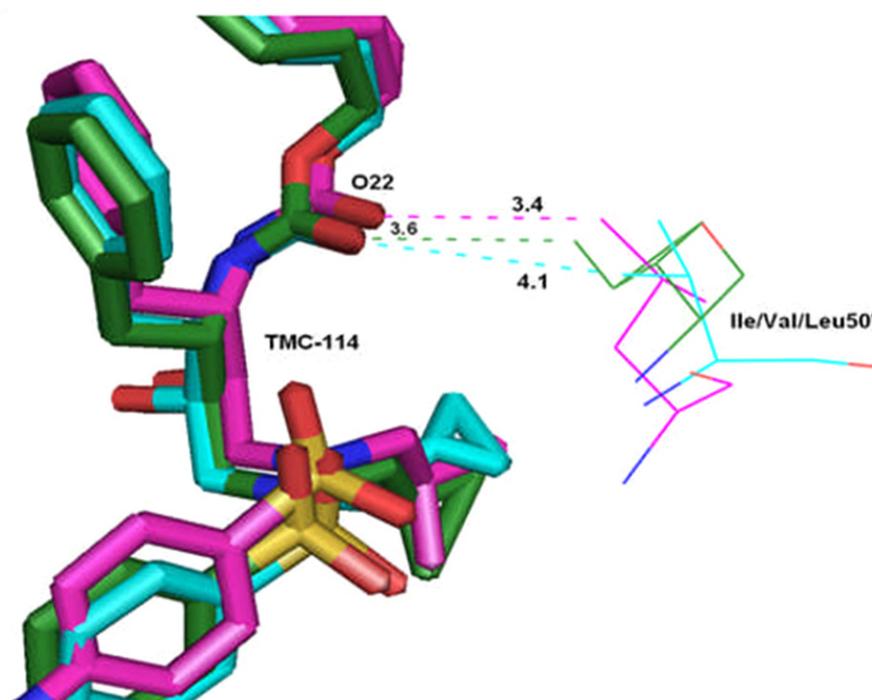
#### *Molecular mechanism of drug resistance*

In the I50L/A71V double mutant, the substitution of isoleucine to leucine, places the methyl group in an altered position, though structure of the side chain is not altered substantially. However, the replacement from alanine to valine adds two methyl groups to the backbone carbon in place of single methyl, which renders side chain bulkier. It is noted that, the change from Ala71 to Val71 in the cantilever region of the HIV-pr has not affected H-bond pattern; however, the H-bond between the residues Arg14' and Glu65' has been reduced (Meher and Wang, 2012). So, it is confirmed that the mutation A71V have no direct influence on the active site conformation and binding affinity. The mutation has allosteric effect on the binding affinity with change in the mobility of active site residues (Fig. 13). The resultant alteration in the conformation of the enzyme may affect its binding affinity to the inhibitor TMC114 to the protease.

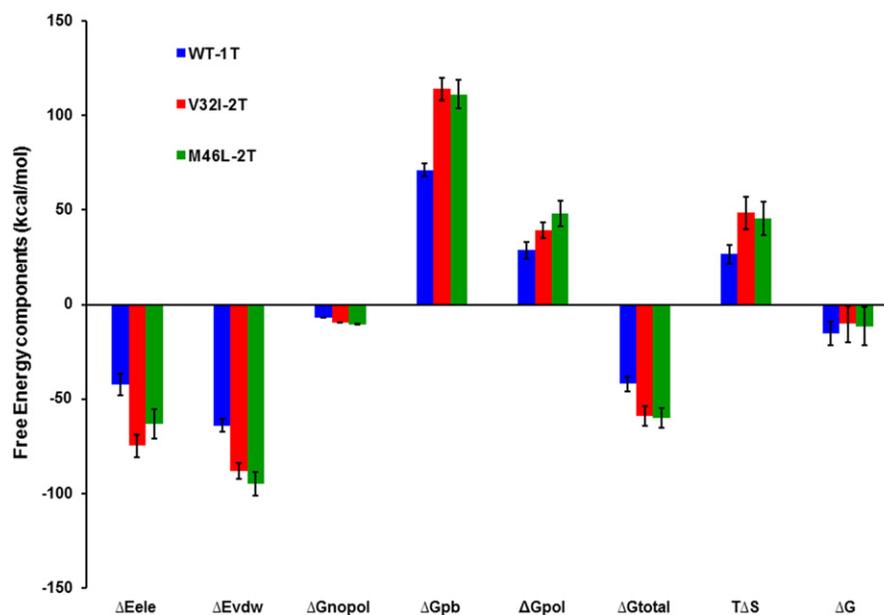
In the I50V mutant HIV-pr, the replacement of isoleucine with valine leads to the loss of a methyl group. It lowers the contact with the central phenyl of TMC114, which leads to the decrement in the van der Waals energy between Val50 and TMC114. On the contrary, for Val50' the change displays a substantial decline in van der Waals energy (by 0.54 kcal/mol), which may be due to the falling of C-H...O interactions between the Val50' side chains and the O22 of TMC114. Fig. 14 confirms that the distance of C...O22 (3.4 Å) for I50V-HIV-pr is longer than that for WT and I50L/A71V-HIV-pr (4.1 and 3.6 Å, respectively), which is likely to be the reason for the less binding affinity and drug resistance.

#### **V32I and M46L mutations effect on TMC114 binding**

Meher and Wang (2015) investigated the binding of inhibitor TMC114 (Fig. 5) to WT, V32I mutant, and M46L mutant HIV-pr with all-atom MD simulations as well as MM-PBSA calculation. The analysis describes the resistance profile of both the mutants (V32I and M46L) with their 1T (single TMC114 bound alone to the active site region) and 2T (bound to the flap and active site region simultaneously) forms. The average flap-flap distance and flap tip-active site distances are longer for the M46L-2T HIV-pr complex as compare to the WT-1T and V32I-2T complexes suggesting an increased flexibility in M46L-2T, feasibly making the



**Fig. 14** C-H...O interactions between the inhibitor TMC114 and the flap region amino acids (Gly49, Gly40', Ile/Val/Leu50, and Ile/Val/Leu50'). TMC114 in stick form is colored by the atom type, and residues are denoted as lines (green-WT; cyan-I50V; purple-I50L/A71V). Reprinted from Meher, B.R., Wang, Y., 2012. Interaction of I50V mutant and I50L/A71V double mutant HIV-protease with inhibitor TMC114 (darunavir): Molecular dynamics simulation and binding free energy studies. *Journal of Physical Chemistry B* 116, 1884–1900. With permission from J. Phys. Chem. B and publisher American Chemical Society (ACS).



**Fig. 15** Energy components (kcal/mol) for the binding of TMC114 to the WT-1T, V32I-2T, and M46L-2T: Eele, electrostatic energy in the gas phase; Evdw, van der Waals energy; Gnp, nonpolar solvation energy; Gpb, polar solvation energy; Gpol = Gele + Gpb; TS, total entropy contribution; H = Eele + Evdw + Gnp + Gpb; ΔG = ΔH - TΔS. The error bars refer to standard deviations (Std.). Reprinted from Meher, B.R., Wang, Y., 2015. Exploring the drug resistance of V32I and M46L mutant HIV-1 protease to inhibitor TMC114: Flap dynamics and binding mechanism. *Journal of Molecular Graphics and Modelling* 56, 60–73. With permission from J. Mol. Graph. Mod. and publisher Elsevier.

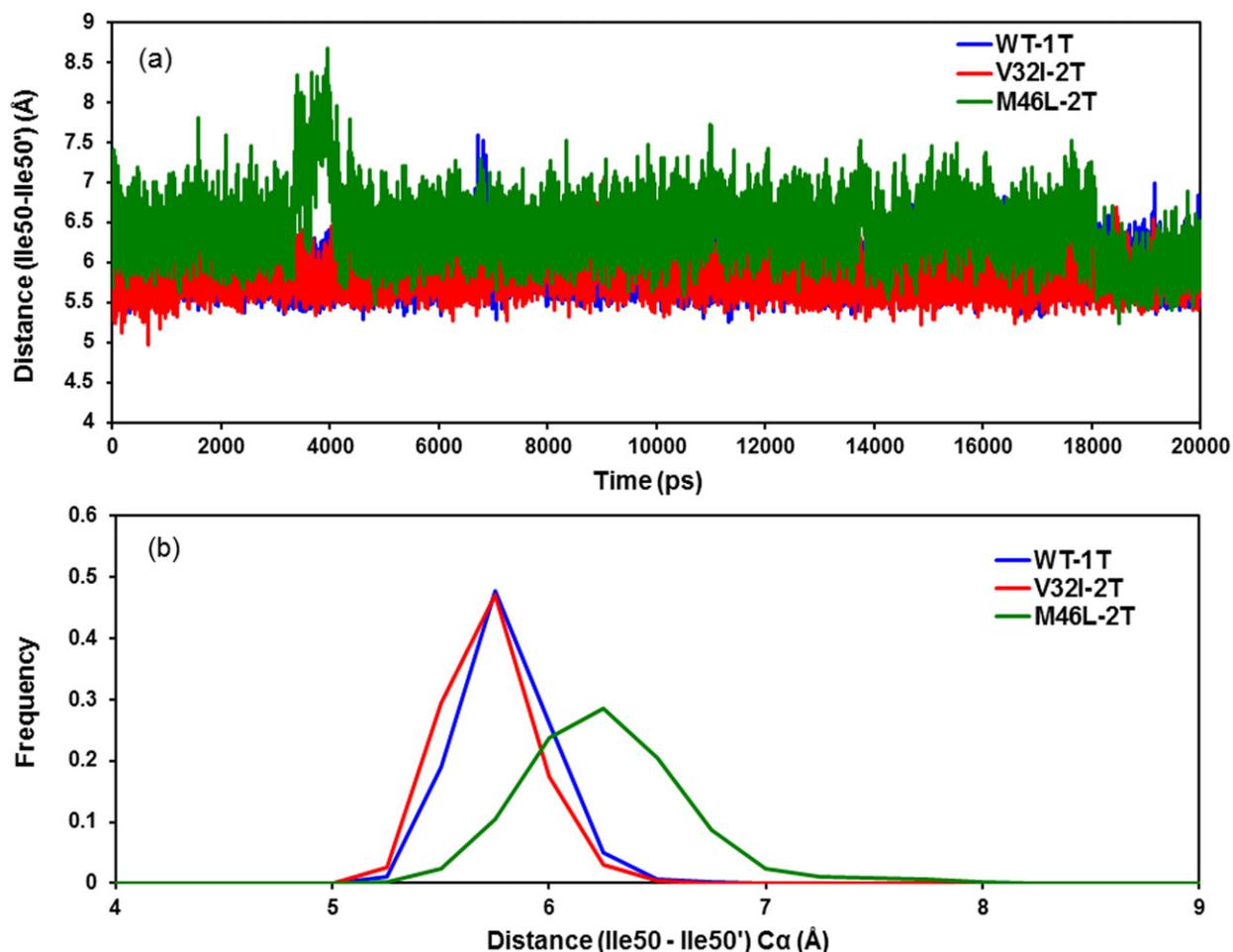
active site capacity larger. From the binding free energies of all the complexes, it was gathered that both the 1T and 2T forms of the protease HIV-pr mutants show resistance to the inhibitor TMC114. Also, it came forth that the binding of the TMC114 on the flap region has inconspicuous impact on the binding affinity (Fig. 15).

#### Comparing the complexed form of protein WT vs. Mutant

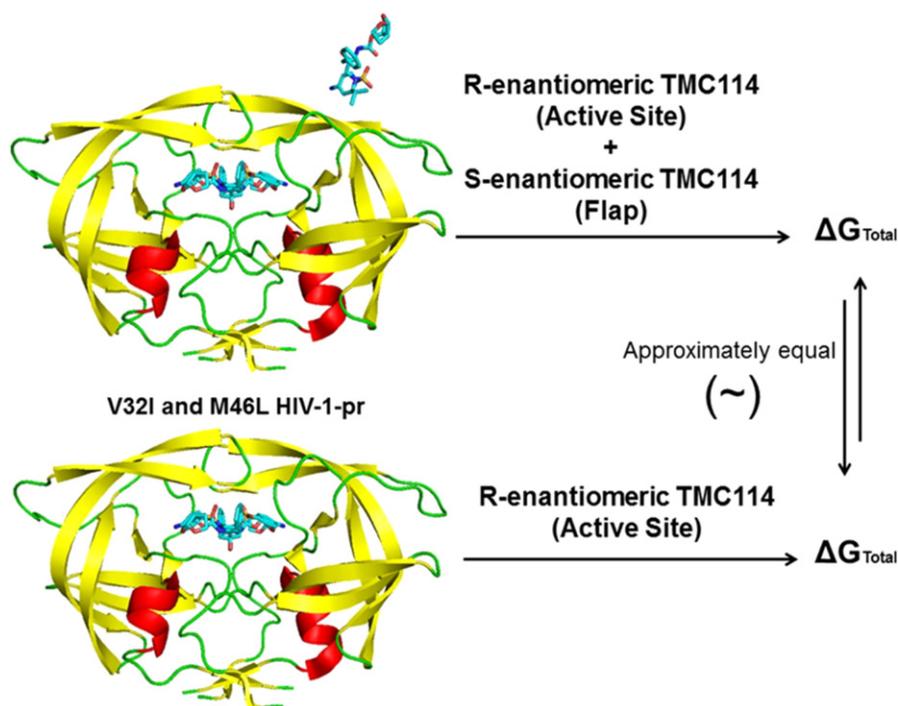
The difference of RMSF (root mean square fluctuations) for the whole protein in its complexed form shows that the two mutations (V32I and M46L) cause more conformational changes of the HIV-pr near the flap elbows, fulcrum (Trp6, Ile15-Gly16 and Glu35-Lys41 & Trp6', Glu35' and Arg57'), and cantilever regions (Pro63-His69 and Ala67'), than the WT counterpart. To explore the relative motion of the flap tips, flap tip-flap tip distance was examined, where, the variation between the double bound and single bound V32I-2T and M46L-2T/HIV-pr was found to be different, the former being broader (Fig. 16). Further studies on flap dynamics studies revealed that there is a floppy flaps movement in M46L-2T in comparison to WT-1T and V32I-2T/HIV-pr structures. Flap RMSD analysis indicates that the binding of TMC114 on the flap -B of the mutant structures has only subtle effect on the flap dynamics.

#### Molecular mechanism of drug resistance

V32I mutation can lead to drug resistance by affecting the interactions between the amino acid side chains and the inhibitor. In the V32I-mutant HIV-pr, the substitution of valine with isoleucine leads to a gain of methyl group. It increases the interaction with the central phenyl of TMC114, and possibly increases the steric hindrance (unfavorable interactions), resulting in a reduced binding affinity to TMC114. This change results in an increase in total entropy contribution ( $T\Delta S$ ) by about 3.42 kcal/mol for V32I-1T and 20.89 kcal/mol for V32I-2T as compared to the WT-1T/TMC114 HIV-pr complex (Meher and Wang, 2015). M46L mutation does not impact the inhibitor binding the active site region, but the atoms of Met46 residue may be forming H-bonds



**Fig. 16** (a) Time-series plot and (b) frequency distribution plot for the distance between the flap tip (Ile50-Ile50) C atoms for the double bound TMC114 to HIV-1-pr mutants and single bound to WT. Reprinted from Meher, B.R., Wang, Y., 2015. Exploring the drug resistance of V32I and M46L mutant HIV-1 protease to inhibitor TMC114: Flap dynamics and binding mechanism. *Journal of Molecular Graphics and Modelling* 56, 60–73. With permission from *J. Mol. Graph. Mod.* and publisher Elsevier.



**Fig. 17** Comparative schematic view showing the binding of TMC114 in the allosteric site (flap region) for mutants (V32I-2T and M46L-2T) does not influence the binding affinity of the system, which can be compared with the mutants (V32I-1T and M46L-1T). Reprinted from Meher, B.R., Wang, Y., 2015. Exploring the drug resistance of V32I and M46L mutant HIV-1 protease to inhibitor TMC114: Flap dynamics and binding mechanism. *Journal of Molecular Graphics and Modelling* 56, 60–73. With permission from J. Mol. Graph. Mod. and publisher Elsevier.

with substrate analogs (Tie *et al.*, 2005). Hence, M46L mutation can have effect on binding affinity indirectly via the weakened hydrophobic interactions (Kovalevsky *et al.*, 2006a). This alteration results in an increase in total entropy contribution ( $T\Delta S$ ) by about 2.64 kcal/mol for M46L-1T and 16.83 kcal/mol for V32I-1T as compared to the WT-1T/TMC114 HIV-pr complex (Meher and Wang, 2015). Therefore, the entropy penalty ultimately compresses the binding affinities and elicit the drug resistance for the V32I and M46L mutations. However, binding of TMC114 in the allosteric site (flap region) does not contribute much in the total gain in binding affinity of the system, due to significant entropy loss leading to the lower binding free energies (Fig. 17).

## Future Directions

The synergy between mutation and conformational dynamics has exposed the mechanisms of drug resistance. There are potential directions that can be discovered on the basis of the current work.

- 1) Information regarding the placing of a larger group at the P2' position of JE-2147 and also replacement of a group at the position of O18 in the TMC114 structure will be helpful in drug designing. One can proceed for the structural improvement of TMC114 and JE-2147 in vitro and in silico as well based on the combinatorial chemistry.
- 2) Binding of the inhibitor (TMC114) in the flap region does not improve the binding affinity of the system. This understanding will promote the development of drugs/inhibitors targeting mostly to the active site region and not to the flap region. However, targeting to other important regions like flap elbow and fulcrum may help in development of allosteric site inhibitors, which may promote the total gain in binding free energies of the system.

## Concluding Remarks

MD simulation has been a tool of tremendous importance and offers generous understandings into the mechanistic features of macromolecular 3D-conformation and dynamics at atomic level. It illuminates the mechanisms of drug binding and resistance profile towards HIV-pr. The study steered to the outcome that 3D-conformational dynamics of HIV-pr is affected by the change in 3D-data coordinates in the protein crystal structure due to mutations that have an important role on HIV-pr conformation and dynamics. Conclusively, the understanding of the molecular basis of drug resistance is a difficult job. However, the result of this study as well as other prior revisions on HIV-pr may offer insightful knowledge with which to design favorable and potent drugs/inhibitors.

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