doi: 10.4149/gpb_2016028

Comparative molecular dynamics study of dimeric and monomeric forms of HIV-1 protease in ligand bound and unbound state

Monikaben Padariya and Umesh Kalathiya

Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdansk University of Technology, Narutowicza St 11/12, 80-233 Gdansk, Poland

Abstract. Human immunodeficiency virus type 1 protease is a viral-encoded enzyme and it is essential for replication and assembly of the virus. Inactivation of HIV-1 protease causes production of immature, noninfectious viral particles and thus HIV-1 protease is an attractive target in anti-AIDS drug design. In our current work, we performed molecular dynamics (MD) calculations (500 ns) for two different ligands (COM5 – designed in our previous study, and Darunavir) and made effort to understand dynamics behaviour of our designed compound COM5. An apo form of HIV-1 protease as monomer and dimer form was also studied in order to analyze response of protein to the ligand. MD results suggest that presence of ligand in hinders the stability of HIV-1 protease and one monomer from dimer systems is dominant on other monomer in terms of interaction made with ligands. We were able to trace functional residues as well as continuous motion of opening and closing (clapping) of flap region in HIV-1 protease (apo form) during entire 1000 ns of MD simulation. COM5 showed almost similar behaviour towards HIV-1 protease enzyme as Darunavir and propose as promising lead compound for the development of new inhibitor for HIV-1 protease.

Key words: HIV-1 — Protease — Molecular dynamics — Darunavir — Inhibitors

Introduction

The human immunodeficiency virus type 1 protease is a viral-encoded enzyme that forms a homodimer. In HIV-1 as in all retroviruses production of infectious virus invariably requires an active viral protease (Flexner 1998). HIV-1 currently infects 35.3 million people estimated worldwide and the number of infected people and death due to AIDS continue to rise despise the availability of antiviral drugs (UNAIDS 2013). HIV-1 protease is essential for replication and assembly of the virus and inactivation of HIV-1 protease enzyme causes production of immature, noninfectious viral particles and thus HIV-1 protease is an attractive target in anti-AIDS drug design (Kohl et al. 1988; Flexner 1998). Much of the improvement in HIV-1 related clinical outcomes has been associated with the use of protease inhibitors in combination with antiretroviral therapy (Palella et al. 1998). Thus, the idea of inhibiting viral replication by disturbing function of protease has led to the development of a class of drugs known as protease inhibitors (Katoh et al. 1987).

HIV-1 protease exists as a homodimer, with each subunit made up of 99 amino acids and this was investigated using X-ray crystallography (Davies 1990). The active site lies between identical subunits and has the characteristic Asp-Thr-Gly (Asp25, Thr26, and Gly27) sequence common to aspartic proteases (James and Sielcki 1983). Two Asp25 residues (one from each monomer or chain) act as the catalytic residues (James and Sielcki 1983) and the conserved active site residues form a symmetrical and highly hydrogen-bonded arrangement (Moore and Stevenson 2000). According to the mechanism of HIV-1 protease protein cleavage proposed by Mariusz Jaskolski

Electronic supplementary material. The online version of this article (doi: 10.4149/gpb_2016028) contains supplementary material, which is available to authorized users.

Correspondence to: Monikaben Padariya, Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdansk University of Technology, Narutowicza St 11/12, 80-233 Gdansk, Poland
E-mail: monika.padariya@pg.gda.pl; monikapadariya1@gmail.com

and colleagues, water acts as a nucleophile, which acts in simultaneous conjunction with a well-placed aspartic acid to hydrolyze the scissile peptide bond (Jaskólski et al. 1991). Moreover, HIV-1 protease has two molecular "flaps" which move a distance of up to 7 Å when the enzyme comes in contact with a substrate (Miller et al. 1989). HIV-1 protease inhibitors work by binding to the active site and essentially becoming "stuck", disabling the enzyme. This results in the production of immature proteins that cannot assemble into infectious virions (Rang et al. 2007).

Understanding topology of HIV protease is heavily based upon one specific hypothesis of the mechanism governing flap motion. The possible convention for terminology of the topology of HIV-1 protease that is independent of all mechanistic hypothesis is as follows: The Flap = residue 43–58, the Ear or Ear Flap = residue 35–42 (also called as the Elbow of the Flap), the Cheek Turn = residue 11–22 (also called as the Fulcrum), the Cheek Sheet = residue 59–75 (also called as the Cantilever), the Eye = residue 23–30 (contains the catalytic Asp that "sees" the drug), and the Nose = residue 6–10 (blocks the front entrance to the active site). Likewise, the Whiskers (residue 1–5 and 95–99) would refer to the termini involved in forming dimerization interface, residue 86–90 form the helix, and residue 79–84 form the Wall Turn (the turn composing the active site's wall) (Perryman et al. 2004) (Fig. S1).

Protease inhibitors that were developed initially had poor oral bioavailability and were administered through intravenous infusion. Improvements in the solubility of agents enhanced oral bioavailability to allow for larger-scale clinical development. Inhibitors that have been approved by the US Food and Drug Administration (FDA) for the treatment of HIV-1 infection routinely are: Saquinavir (SQV) (Brower et al. 2008), Ritonavir (RTV) (Wlodawer 2002), Indinavir (IDV) (Wlodawer 2002; Flexner 2007), Nelfinavir (NFV) (Wlodawer 2002), Amprenavir (APV) (Wlodawer 2002; Flexner 2007), Lopinavir (LPV) (Wlodawer 2002; Flexner 2007), Fosamprenavir (FAV) (Chapman et al. 2004; Flexner 2007; Luber et al. 2007), Atazanavir (ATZ) (Yanchunas 2005; Flexner 2007), Tipranavir (TPV) (Flexner 2007; Schobert et al. 2008), and Darunavir (DRV) (Flexner 2007; McCoy 2007). These protease inhibitors approved by FDA have demonstrated potent antiretroviral activity and clinical benefits. However, they have limitations individually and collectively which involves (for some or all of the currently available protease inhibitors) bioavailability, large pill numbers, dosing frequency, dosing schedule with meals, and toxicity. Potential cross-resistance within available protease inhibitors is a serious concern, and the success of salvage treatment of patients whose treatment with a protease inhibitor has failed is by no means guaranteed. Further development of additional protease inhibitors that address some or all of these issues is essential (Eron 2000).

Structure-assisted design of HIV-1 protease inhibitors is an iconic example of rational drug design (Wlodawer and Vondrasek 1998). The first generation protease inhibitors (approved before 2000) comprised peptidomimetics designed to mimic the scissile bonds of natural protease substrates. Their use is linked to the rapid emergence of resistance, low drug availability, toxicity and various side effects. Considering these aspects, pharmaceutical companies and academia began to develop second-generation inhibitors to overcome these limitations (Kožíšek et al. 2014).

In our previous study (Kalathiya and Padariya 2014) regarding HIV-1 inhibition, we have designed a series of ligand molecules using structure-based design approach and tested towards HIV-1 protease, reverse transcriptase, and integrase enzymes using flexible and rigid docking methods. Main scaffold structure for HIV-1 inhibitors was designed considering importance of benzyl and imidazole groups to the HIV-1 enzyme inhibitors in our previous work (Kalathiya and Padariya 2014). Moreover, reverse design approach was applied to design new compounds, instead of taking an already available inhibitor and adding functional groups to it to improve its potency, a novel new scaffold was designed and important functional groups were taken from some of already approved compound by FDA and they were added in designed scaffold. Designed scaffold was modified to obtain series of HIV-1 inhibitors by adding potential functional groups of FDA approved drugs to improve its binding affinity towards selected key enzymes of HIV-1 (Kalathiya and Padariya 2014).

For flexible docking (CDOCKER), only active site residues were considered and for rigid docking (AutoDock), monomeric form of HIV-1 protease was taken into account in our previous study (Kalathiya and Padariya 2014). Among tested compounds (Kalathiya and Padariya 2014), COM5 had obtained best binding towards HIV-1 protease and considering those results, we have analyzed two different forms (monomer and dimer) of HIV-1 protease containing COM5 in our present study, to understand protein-ligand molecular details. Chemical structure of COM5 (Kalathiya and Padariya 2014) contains main scaffold and two functional groups derived from IDV, an FDA-approved HIV-1 inhibitor (Wlodawer 2002; Flexner 2007). Apo-form of monomer and dimer structure of HIV-1 protease was also considered in this study in order to characterize response of protein towards inhibitor (Fig. 1). Moreover, in our previous work we had used molecular docking approaches which provide static mode of interaction between two molecules whereas in this work, we have used molecular dynamics (MD) approaches that gives dynamic behavior of protein towards inhibitor or ligand molecule.

Apart from COM5 which was designed in our previous study about HIV-1 reverse transcriptase, protease and integrase (Kalathiya and Padariya 2014), one more inhibitor

studied in this work was FDA-approved HIV-1 protease inhibitor Darunavir (MacArthur 2007). Darunavir (DRV, also known as TMC-114, UIC-94017) was a potent second-generation HIV protease inhibitor marketed under the name Prezista (Johnson & Johnson, New Brunswick, NJ, USA) since 2006. Darunavir was recently approved by FDA as HIV-1 protease inhibitor (MacArthur 2007) and has shown to be less susceptible to viral resistance than other previously approved HIV-1 protease inhibitors (Ghosh et al. 2007; Madruga et al. 2007). Even though Darunavir was chemically related to APV, it binds nearly 100-times more tightly than APV and 1000-times more tightly than SQV, IDV, RTV and NFV to the wild-type HIV-1 protease (King et al. 2004; Dierynck et al. 2005). It was on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system (WHO 2015).

Darunavir received attention at the time of its release, as it serves a treatment option for people with drug resistant HIV. Darunavir was a nonpeptidic inhibitor of protease that stuck itself in the active site of protease by forming a number of hydrogen bonds (Leonis et al. 2012). It was developed to increase interactions with HIV-1 protease and to be more resistant against HIV-1 protease mutations. Darunavir has a much stronger interaction with protease with a K_d value of 4.5×10^{-12} M (King et al. 2004). This strong interaction comes from increased hydrogen bonds between Darunavir and the backbone of protease active site. Structure of Darunavir allows it to create more hydrogen bonds with the protease active site than most protease inhibitors that have been developed and approved by the FDA (Lefebvre and Schiffer 2008). Moreover, the backbone of HIV-1 protease maintains its spatial conformation in presence of mutations and as Darunavir interacts with this stable portion of protease, the enzyme-inhibitor interaction was less likely to be disrupted by a mutation (Lascar and Benn 2009). Darunavir interacts with catalytic aspartates (Asp25 of each monomer) and backbone of active site through hydrogen bonds, specifically binding to residues Asp25 and Asp30 from both monomers as well as with Gly27 and Asp29 residues. These interactions prevent viral replication, as it competitively inhibits the viral polypeptides from gaining access to the active site and strongly binds to enzymatic portions of this protein (Leonis et al. 2012).

The effect of binding two different inhibitors (Darunavir and COM5 – our designed ligand in previous study) on the protease structure is currently the focus of intensive research. Here, we employed molecular dynamics simulations to analyze the structural stability of HIV-1 protease in two different forms, as monomer and dimer. Six independent systems were generated and studied for comparative analysis which involves: (i) HIV-1 protease monomer in absence of ligand (apo-form), (ii) HIV-1 protease dimer in absence of ligand (apo-form), (iii) HIV-1 protease monomer in complex with Darunavir inhibitor, (iv) HIV-1 protease dimer in complex with Darunavir inhibitor, (v) HIV-1 protease monomer in complex with COM5, and (vi) HIV-1 protease dimer in complex with COM5.

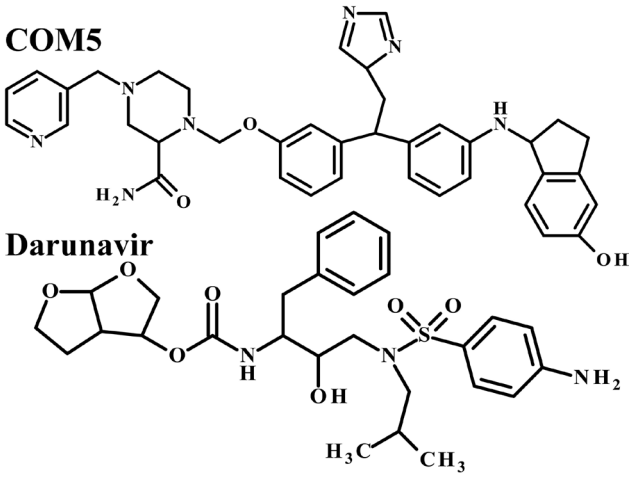

Figure 1. Chemical structure of COM5, selected from our previous work concerning HIV-1 enzymes (Kalathiya and Padariya 2014) **(A)** and one of the FDA-approved HIV-1 protease inhibitor Darunavir (MacArthur 2007) **(B)**.

X-ray crystallography has considerably contributed to the discovery of HIV-1 protease inhibitors (Wlodawer and Vondrasek 1998). More than 400 structures of this enzyme in complex with various inhibitors have advanced insights into inhibitor binding modes (Weber and Agniswamy 2009; Ali et al. 2010). In our study, we have chosen very recently deposited crystal structure of wild-type HIV-1 protease in complex with Darunavir (PDB ID: 4LL3) having resolution of 1.95 Å (Kožíšek et al. 2014). This MD simulation study was initiated by the questions: (i) Was HIV-1 protease structurally stable as in monomer form? (ii) Is the stability of binding mode of inhibitor in different studied complexes similar or not? (iii) Whether the Darunavir as well as inhibitor COM5 binds to monomeric HIV-1 protease or not? (iv) How these inhibitors binds to the two different forms (monomer and dimer) of HIV-1 protease? and (v) does it explain the mechanism of inhibition? (vi) How COM5 inhibitor response to HIV-1 protease monomer and dimer during MD simulation?

Materials and Methods

Protein and ligand preparation

Crystal structure (PDB ID: 4LL3) (Kožíšek et al. 2014) of HIV-1 protease was retrieved from RCSB Protein Data

Bank (Berman et al. 2000) with resolution of 1.95 Å in complex with ligand Darunavir (Kožíšek et al. 2014). Initially all internal ions and waters were removed. Total six different systems were generated using crystal structure of HIV-1 protease: (i) HIV-1 protease dimer with Darunavir, (ii) HIV-1 protease monomer with Darunavir, (iii) HIV-1 protease dimer with COM5, (iv) HIV-1 protease monomer with COM5, (v) HIV-1 protease dimer without any ligand (apo form), (vi) HIV-1 protease monomer without ligand (apo form). For systems with Darunavir, coordinates of ligand were kept as they were in the original PDB file and for systems with ligand COM5 coordinates of ligand were retrieved from CDOCKER results obtained from our previous studies (Kalathiya and Padariya 2014).

Molecular dynamics simulation using GROMACS

MD calculations were performed for all six systems using GROMACS software (Lindahl et al. 2001). GROMACS is a versatile package that uses Newtonian equations of motion over hundreds to millions of particles during MD calculations. GROMACS along with speed of computing it supports different algorithms (Lindahl et al. 2001; Hess et al. 2008). For systems with ligands Darunavir or COM5 molecular dynamics was carried using GROMACS 4.6.5 (Hess et al. 2008) package with GROMOS96 43A1 (Schuler et al. 2001) forcefield.

Selection of forcefield for molecular dynamics

The work done by Elio et al. (2012) described that comparatively, CHARMM27 was less effective to clearly characterize the folding events occurring in protein structure while GROMOS96 43a1 performed better in a given specific time. CHARMM27 tends to be rigid to some extent and makes the protein structure more conserved (may require more computational time), where as GROMOS96 43a1 provide flexibility to a protein structure to obtained a defined structure within less computational time. This type of flexibility might be useful when studying protein with ligand systems, as in our study we were interested in studying the dynamics behavior of HIV-1 protease with different ligands. Furthermore, GROMOS96 43a1 forcefield are united atom forcefield and CHARMM27 an all-atomic forcefield, all-atomic forcefield (simulations) may require more computational time for studying folding, conformational changes, and allosteric transitions. Work done by Levy et al. (2004) concluded that monomeric HIV-1 protease is stable and its folding is conditional for the dimer formation. Besides this, experimental denaturation studies suggested that the folding and binding of HIV-1 protease occurs simultaneously (Grant et al. 1992; Todd et al. 1998; Xie et al. 1999). Considering all these aspects and not to limit this kind of events in structure of HIV-1 protease upon ligand bound and unbound (apo) form during our MD study, we decided to use GROMOS96 43a1 forcefield for ligand bound system for 500 ns of MD simulation and CHARMM27 forcefield to study apo form of protein by 1000 ns of MD simulation.

Additionally, both GROMOS96 43a1 and CHARMM27 forcefield were tested for HIV-1 protease monomer apo systems by performing 200 ns of MD simulation (without ligand). Results obtained from MD suggest that overall structure of protein tends to be more stable/less flexible in CHARMM27 forcefield environment, except N and C terminal of HIV-1 protease, as compared to that of GROMOS96 43a1 forcefield (Fig. S2). Looking over the root mean square deviation (RMSD) values of HIV-1 protease (monomer form) in both forcefield, it was observed that monomer was highly fluctuating in CHARMM27 as compared to GROMOS96 43a1 forcefield, and the RMSD value reached till ~6.5 Å. But when calculating RMSD excluding N terminal residues (residue number 1–8) of HIV-1 protease, protein has shown almost same and stable behavior in both forcefield (Fig. S2). GROMOS96 43a1 forcefield was used for ligand bound system in order not to limit flexibility in ligand-protein complex during MD simulation and CHARMM27 forcefield to study unbound form (apo) of protein to kept protein in a comparatively slight rigid (compact) environment.

Molecular dynamics system setup

Initially both HIV-1 protease ligand bound and unbound (apo form) systems were hydrated using water model of SPC type. Following water addition to systems, periodic boundary conditions (PBC) were applied in all xyz directions. The periodic box was set to be as rhombic dodecahedron model, and distance between system and periodic box was set to 1 nm. NaCl (salt) concentration of 0.15 M was added to HIV-1 protease ligand bound and unbound systems, to neutralize them.

To minimize energy of systems, 5000 steps of steepest descent were applied. Minimization of systems was then followed by equilibration (1000 ps) of systems. During equilibration of systems heavy atoms were restrained in order to allow adjustment of water molecules with counter ions. Systems in equilibration phase were heated at constant temperature under isothermal-isobaric conditions NPT (number of particles (N), system pressure (P), and temperature (T)) ensemble and Particle-Mesh-Ewald (PME) method (Darden et al. 1993) was used to treat the electrostatic interactions. Bonds between heavy atoms and complementary non-polar hydrogen atoms bonds were constrained to their equilibrium bond lengths using the LINCS algorithm (Hess et al. 1997). Temperature was kept constant (300 K) and pressure was maintained

at 1 bar using V-rescale thermostat (Bussi et al. 2007) and Parrinello-Rahman barostat (Parrinello and Rahman 1981), respectively. Production run was carried out for 500 ns of leap-frog integrator (van Gunsteren and Berendsen 1988) and the coordinate data were written to file every 10 ps for systems with respective ligands COM5 or Darunavir. For systems without ligand, MD calculations were performed for 1000 ns using the GROMACS (Hess et al. 2008) simulation software package with CHARMM27 (Bjelkmar et al. 2010) forcefield and coordinates obtained from MD calculations were saved for every 10 ps.

Results

In order to understand behaviour and stability of HIV-1 protease enzyme with our compound COM5 (Kalathiya and Padariya 2014), it was studied with monomer and dimer form of HIV-1 protease enzyme. An already available inhibitor Darunavir (Kožíšek et al. 2014) was also tested with same methodology as well as in dimer and monomer form of HIV-1 protease to understand behaviour of HIV-1 protease in presence of different ligands. Additionally for making comparative analysis, HIV-1 protease enzyme was also studied without any ligand (apo form) as monomer and dimer form using molecular dynamic approach. Results obtained from MD trajectories for different systems were compared with each other and dynamic behaviour of ligands with protein was analyzed.

Structural stability of HIV-1 protease enzyme and ligands

Root mean square deviation (RMSD)

RMSD values were calculated from MD trajectories to understand the stability of a system and to determine quantitatively extent of motion in protein and ligands. From analysis of RMSD values of HIV-1 protease and ligand COM5 complex, we found that in system with dimer form of protein with ligand both monomer (Chain A and Chain B) remained steady till 120 ns and in the following simulation time Chain B had more fluctuation as compared to chain A, this fluctuation could be termed as the change in behaviour of protein towards ligand COM5 (Fig. 2). Comparing Chain A from monomer and dimer systems, it was observed that Chain A from monomer system has more fluctuation as compared to that from dimer system, indicating second monomer Chain B in dimer system plays role in the stability of Chain A (Fig. 2).

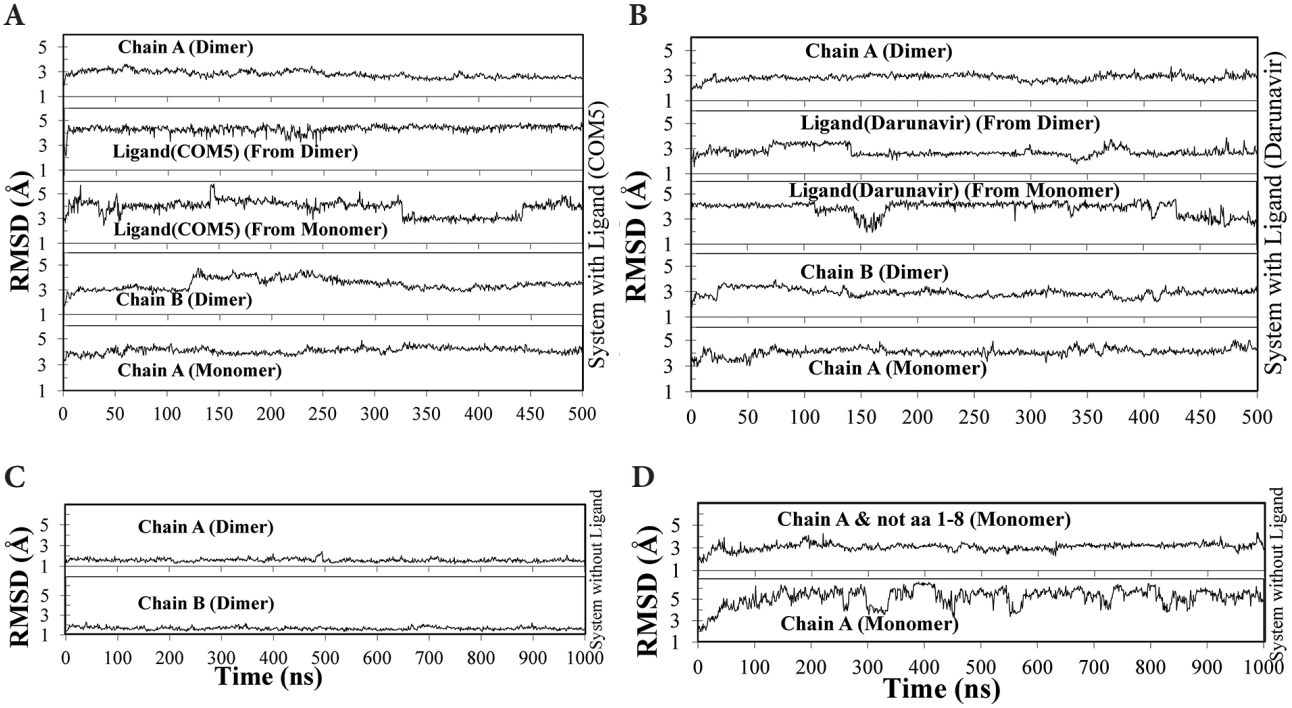

Figure 2. RMSD plots for HIV-1 protease enzyme (PDB ID: 4LL3) (Kožíšek et al. 2014) in complex with/without ligand. **A.** Dimer and monomer form of HIV-1 protease enzyme with ligand COM5. **B.** Dimer and monomer form of HIV-1 protease enzyme with ligand Darunavir. **C.** Dimer form of HIV-1 protease enzyme without ligand. **D.** Monomer form of HIV-1 protease enzyme without ligand, and monomer RMSD excluding highly fluctuating N terminal residues (Pro1-Arg8).

Analyzing RMSD of ligand COM5, it was observed that ligand in complex with monomer form had more fluctuation as compared to that of dimer system. For systems with ligand Darunavir similar type of behaviour of protein in dimer and monomer form was observed, but with less fluctuation in RMSD values as compared to that of systems with ligand COM5. Observing RMSD values of systems without any ligand states that HIV-1 protease was more stable in dimer form (apo form) as compared to that in complex with ligand (COM5 or Darunavir), suggesting that ligand either hinders the stability of HIV-1 protease enzyme (Fig. 2). Opposite behaviour of monomer form of HIV-1 protease (apo form) was observed as compared to dimer system. In monomer ligand unbound systems, it has been found that N and C terminal residues were highly fluctuating which resulted in sudden jumps in RMSD plots, computing RMSD excluding these N terminal residues (Pro1-Arg8) protein showed a stable behavior (Fig. 2). Overall more fluctuation in RMSD values were observed in apo form of monomer system, as compared to monomer form with ligand COM5 or Darunavir, thus ligand or another monomer was required for the stability of HIV-1 protease enzyme (Fig. 2).

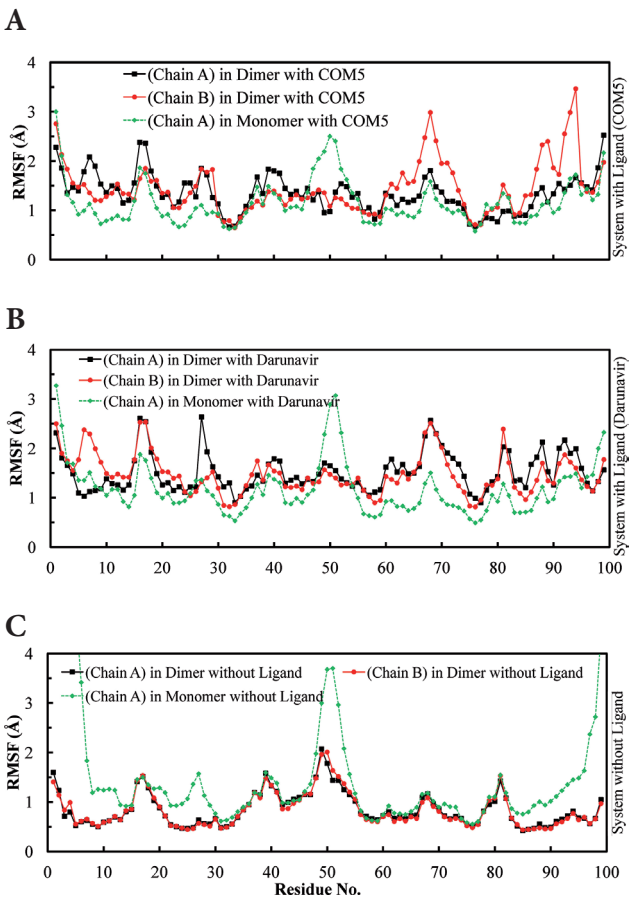

Figure 3. RMSF values of HIV-1 protease enzyme (PDB ID: 4LL3) (Kožíšek et al. 2014) from all different systems. **A.** RMSF of dimer/monomer form of HIV-1 protease enzyme with ligand COM5. **B.** RMSF of dimer/monomer form of HIV-1 protease enzyme with ligand Darunavir. **C.** RMSF of dimer/monomer form of HIV-1 protease enzyme without ligand.

Root mean squared fluctuation (RMSF)

For analyzing the fluctuation of each residues of HIV-1 protease structure, RMSF were calculated from MD trajectories. Individual HIV-1 protease enzyme residues fluctuation were traced and analyzed based on the region described in (Perryman et al. 2004), nose region (6–10), cheek turn region (11–22), eye region (23–30), ear region (35–42), flap region (43–58), and cheek sheet region (59–75) (Perryman et al. 2004). Figure S2 shows behavior of N and C terminal of HIV-1 protease in two different forcefield, it was observed that in CHARMM27 forcefield, N terminal is floating around whereas in GROMOS96 43a1 forcefield it interacts with protein residues itself and behavior was stable. Analyzing RMSF (root mean squared fluctuation) values of both forcefield, it was observed that each of them behaved same in terms of jumps in residues, comparatively protein residues in CHARMM27 forcefield fluctuated less, except N and C terminals (Fig. S2). This results correlates with intramolecular interactions, as HIV-1 protease in CHARMM27 forcefield has more intramolecular H-Bonds which make protein more compact compared to that of protein in GROMOS96 43a1 forcefield (Figure S2) in apo form. In dimer systems with compound COM5, it was observed that nose region behave opposite in both monomer (Chain A and Chain B), cheek turn of Chain A has higher fluctuation as that of Chain B, cheek sheet and end region of HIV-1 protease for Chain B has higher fluctuation than Chain A (Fig. 3). These residues may be responsible for high RMSD values after 120 ns for Chain B (Fig. 2). In monomer form of HIV-1 protease, flap region had high RMSF values as that of dimer form and rest almost all residues showed lower RMSF values as that of dimer form. Similar as that of dimer system with COM5, in system with ligand Darunavir nose region behave opposite in both monomer (Chain A and Chain B) of dimer form and instead of cheek turn of Chain A, eye region of Chain A has higher fluctuation than that of Chain B.

Monomer system with Darunavir behaved almost similar as that in complex with ligand COM5. In apo systems (without ligand) it was observed that, flap region in dimer form has obtained higher fluctuation than other region of HIV-1 protease enzyme, as well as flaps are more flexible as that of systems with ligand. For apo form of monomer system, end region (N and C terminal residues), eye region, and flap region were more fluctuating where as rest protein residues behaves almost similar as that of dimer apo from

(Fig. 3). Results obtained from our studies correlates well with literature data (Yaakov and Amedeo 2003; Viktor et al. 2006), Levy and Caflisch (Levy et al. 2004) studied apo form of monomer/dimer systems and Viktor et al. (2006) studied ligand bound and free state of HIV-1 protease enzyme. Looking over the RMSF plots obtained for HIV-1 protease from 500 ns (of ligand bound) and 1000 ns (of apo form) of simulation time (Fig. 3), it was observed that flaps have more fluctuation in monomer system compared to dimer where as in work by Levy and Caflisch Levy et al. 2004), flap region from both systems (monomer and dimer) behaved similar which may be due to short simulation time of 20 ns in their work (Yaakov and Amedeo 2003) and such difference in behaviour of flaps was not observed. Our results correlate well with the work done by Viktor et al. (2006), in which flaps region in apo form are more fluctuating as that of ligand bound form. Additionally, in work of Viktor et al. (2006) they performed MD simulation of 42 ns (ligand free) and 28 ns (ligand bound) in which they observed that for flap region in free state, RMSF value reaching up to 4 Å, where as in our work of 1000 ns of MD simulation for apo form RMSF of flap region reached up to 2 Å.

H-Bond interactions and binding mode of COM5/Darunavir with HIV-1 protease

To characterize behaviour of ligands with HIV-1 protease enzyme, interactions (H-Bond) of ligand COM5 and Darunavir with HIV-1 protease were traced throughout MD simulations. Evaluating overall H-Bond formation of ligand COM5 and Darunavir with HIV-1 protease dimer and monomer form (of Chain A), it was observed that monomer form of HIV-1 protease has formed more H-Bonds as compared to dimer form (of Chain A) (Fig. S3). Indicating second monomer (Chain B) actively taking part in interaction with ligand in dimer and both monomers of HIV-1 protease dimer (Chain A and Chain B) were involved in ligand interaction. During MD simulation, it was observed that ligand COM5 and Darunavir formed H-Bonds with HIV-1 protease during entire 500 ns simulation time in dimer and monomer systems (Fig. S3).

To estimate in detail about receptor-ligand interactions of HIV-1 protease/COM5 and HIV-1 protease/Darunavir complex and to examine dynamic stability, we analyzed % of H-Bond occupancy throughout MD simulation time (Table 1). Rapid motion of ligand and protein in MD simulation generated numerous encounters, thus H-Bond showing occupancy ≥ 1% over 500 ns were selected as stable and presented in Table 1. For HIV-1 protease/COM5 complex (dimer form), it was observed that for Chain A, RMSD and RMSF (Fig. 2 and 3) were more stable and residues less fluctuating as compared to Chain B, correlates well with H-Bond formation of COM5 with individual

Table 1. Hydrogen bond occupancy between ligand-protein complexes during MD

	Residue number	Occ. (%)
COM5		
Dimer		
Chain A	Gly48-M	18.56
	Gly49-M	1.60
	Ile50-S	6.59
	Thr80-M	1.40
	Pro81-M	3.39
	Val82-M	1.30
Chain B	Asp30-M	18.66
	Val32-S	9.98
	Leu33-M	2.20
Monomer		
Chain A	Asp25-S	1.30
	Asp29-M	53.29
	Asp30-M	31.44
	Gly48-M	1.10
	Ile50-S	1.50
	Gly51-M	1.00
	Gly52-M	2.60
	Asn83-M	4.59
	Ile84-S	8.08
Darunavir		
Dimer		
Chain A	Asp29-M	1.10
	Ile50-S	1.00
	Pro81-S	6.79
	Val82-M	1.40
Chain B	Val32-S	1.10
	Gly49-M	1.10
	Ile50-S	4.89
	Gly51-M	30.24
	Gly52-M	10.28
	Pro79-M	1.20
	Pro81-S	1.00
Monomer		
Chain A	Asp29-M	6.69
	Asp30-M	14.17
	Val32-S	1.10
	Gly48-M	2.99
	Ile50-M	35.53
	Thr80-S	1.70
	Val82-M	3.49
	Ile84-S	2.59

Ligand COM5 formed 12 H-Bonds with each monomer (Chain A and Chain B) of a dimer protein, and it formed 35 H-Bonds with monomer protein (Chain A). Ligand Darunavir formed 17 H-Bonds with one monomer Chain A of a dimer protein and 25 H-Bonds with second monomer Chain B of a dimer protein, and it form 36 H-Bonds with monomer protein (Chain A). H-Bond showing occupancy ≥ 1% over 500 ns were presented in the table. Occ., occupancy of an individual H-Bond over 500 ns of simulation time; Resid., residue number; M, main chain of aminoacid; S, side chain of an aminoacid.

monomers of dimer system. Though COM5 has formed 12 H-Bonds with Chain A and Chain B monomer each of a dimer protein, Chain A has more stable H-Bond forming residues as compared to Chain B (Table 1). Identical but opposite behaviour was observed in HIV-1 protease/Darunavir complex, for Chain B RMSD and RMSF (Fig. 2 and 3) were more stable and residues less fluctuating as compared to Chain A. Darunavir formed 17 H-Bonds with one monomer Chain A of a dimer protein and 25 H-Bonds with second monomer Chain B of a dimer protein (Table 1).

Different similarities within/between HIV-1 protease/COM5 and HIV-1 protease/Darunavir complex were observed in terms of interacting residues of protein with ligand. In HIV-1 protease/COM5 complex, residues GLY48 (1.10%), ILE50 (1.50%), and ASP30 (31.44%) of monomer system were found to be interacting with ligand in dimer systems (Chain A: GLY48 (18.56%), ILE50 (6.59%), and Chain B: ASP30 (18.66%)). In HIV-1 protease/Darunavir complex, residues ILE50 and PRO81 from both monomer of dimer system were involved in H-Bond formation with Darunavir. ASP29 (6.69%), VAL32 (1.10%), ILE50 (35.53%), and VAL82 (3.49%) of monomer systems with Darunavir complex were found to be interacting with Darunavir in dimer systems (Chain A: ASP29 (1.10%), ILE50 (1.00%), VAL82 (1.40%), and Chain B: VAL32 (1.10%), ILE50 (4.89%)) (Table 1 and Fig. 4). VAL32, ILE50, PRO81, and VAL82 residues from either of monomer Chain A or Chain B from dimer system were found to be common forming H-Bond interactions with ligand COM5 or Darunavir, respectively. And residues ASP29, ASP30, GLY48, ILE50, and ILE84 were commonly involved in H-Bond interaction in monomer systems of HIV-1 protease/COM5 and HIV-1 protease/Darunavir complex, respectively. Comparing these interacting residues from both HIV-1 protease/COM5/Darunavir complexes (Fig. 4) with literature data (Wartha et al. 2005; Moonsamy and Soliman 2013; Ahmed et al. 2014; Kalathiya and Padariya 2014; Kožíšek et al. 2014; Qiu et al. 2014), it was observed that results correlate well. Residues ASP25, ASP29, ASP30, GLY48, and ILE50 either from dimer and monomer system of HIV-1 protease/COM5/Darunavir complex, showed similar H-Bond interactions with work described in (Wartha et al. 2005; Moonsamy and Soliman 2013; Ahmed et al. 2014; Kalathiya and Padariya 2014; Kožíšek et al. 2014; Qiu et al. 2014), respectively. Apart from these protein-ligand interactions (intermolecular), COM5 and Darunavir had showed intramolecular (interactions within ligand) as well as interactions with water molecules (Fig. S4 and S5). Analyzing ligand interaction with water it was observed that, compounds COM5 or Darunavir in complex with monomer or dimer systems of HIV-1 protease equally form interactions with water (Fig. S4). Indicating that ligand molecules have capability of forming H-Bond interactions with HIV-1 protease through water molecules, results correlates well with work described in (Jaskólski et al. 1991). Analyzing intramolecular interactions, it was observed that ligand COM5 or Darunavir forms more intramolecular interactions in dimer systems as compared to that of monomer system, explaining presence of second monomer effects in the conformational changes in ligand COM5 or Darunavir (Fig. S5).

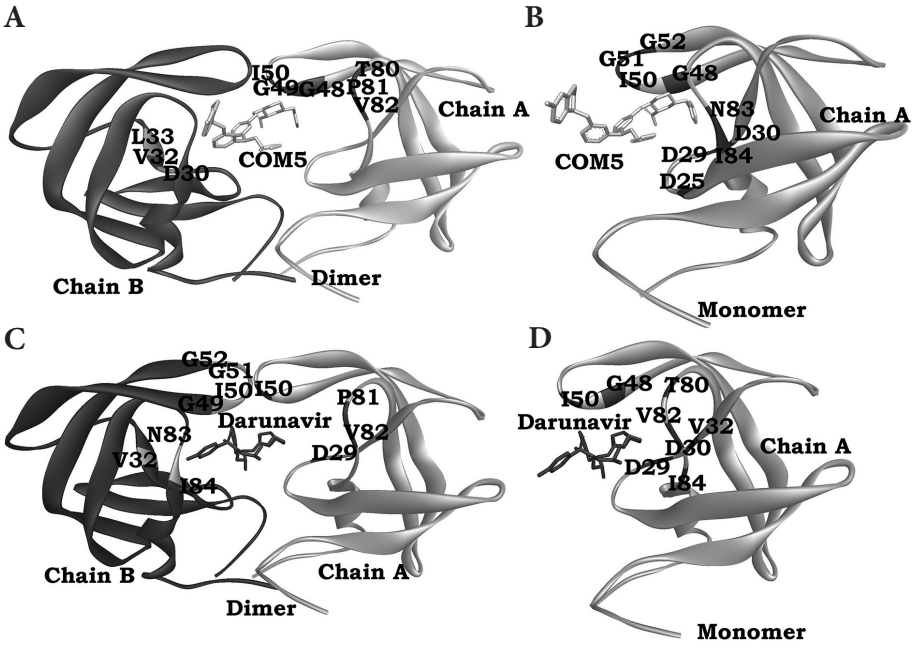

Figure 4. Schematic representation of molecular interaction and binding pattern of ligand COM5 or Darunavir with HIV-1 protease enzyme obtained from Gromacs simulation (Table 1). **A.**,**B.** HIV-1 protease protein with ligand COM5 in dimer and monomer system, respectively. **C.**,**D.** HIV-1 protease protein with ligand Darunavir in dimer and monomer system, respectively. Protein residues from dimer or monomer systems interacting with ligand are labeled in one letter and ID.

Binding affinities for HIV-1 protease/COM5 and HIV-1 protease/Darunavir complex

Interaction energies were calculated to understand receptor-ligand interactions and stability of complex HIV-1 protease/COM5 or HIV-1 protease/Darunavir (Fig. 5 and Table 2). Crude interaction energy based on short-range energy components and a crude qualitative estimate of the stability of the protein-ligand complex was calculated using the formula: $<E_{interaction}> = <E_{Lennard\text{-}Jones}> + <E_{Coulomb}>$. Interaction energy obtained from 500 ns of simulation time for ligand COM5 or Darunavir with HIV-1 protease suggests that, ligands formed more stable complex in dimer system and had more interaction energy as compared to that of monomer systems. Average interaction energy for compound COM5/Darunavir with dimer system was –86.03/–78.03 kcal/mol and for monomer system it was –79.11/–62.48 kcal/mol, respectively. Comparing crude interaction energy obtained from MD for ligand COM5 with Darunavir, it was observed that ligand COM5 has more average interaction energy and stable energies throughout 500 ns of simulation time as compared to that of ligand Darunavir (Fig. 5 and Table 2).

Table 2. Average interaction energy for the HIV-1 protease with COM5 or Darunavir inhibitors obtained from gromacs simulation

Compound	Energie (protein-ligand)	Dimer (kcal/mol)	Monomer (kcal/mol)
COM5	$E_{interaction}$	–86.03	–79.11
	$E_{Lennard\text{-}Jones}$	–78.61	–62.48
	$E_{Coulomb}$	–7.42	–16.63
Darunavir	$E_{interaction}$	–78.03	–61.39
	$E_{Lennard\text{-}Jones}$	–68.74	–52.80
	$E_{Coulomb}$	–9.29	–8.59

$<E_{interaction}> = <E_{Lennard\text{-}Jones}> + <E_{Coulomb}>$, $E_{interaction}$ was a crude interaction energy based on short-range energy components and a crude qualitative estimate of the stability of the protein-ligand complex. $E_{interaction}$, interaction energy; $E_{Lennard\text{-}Jones}$, Lennard-Jones-SR interaction energy; $E_{Coulomb}$, Coulomb-SR interaction energy.

Hydrogen bond interactions of HIV-1 protease

To understand the behaviour of HIV-1 protease protein in systems with and without ligands, inter-intra molecular interactions were studied obtained from MD trajectories. Continuous in motion, residues of protein during MD simulation generated various temporary protein-protein interaction but interaction which showed H-Bond occupancy ≥ 10% over 500/1000 ns, respectively were represented in the Table S1. Residues either from monomer Chain A or Chain B of dimer systems involved in H-Bond protein-protein interaction are represented in table as donor or acceptor residues. HIV-1 protease dimer system in complex with ligand COM5 formed 122 H-Bonds, from which 16 hydrogen bond interactions lasted ≥ 10% of simulation time. HIV-1 protease dimer system in complex with ligand Darunavir formed 105 hydrogen bonds, from which 18 hydrogen bond interactions lasted ≥ 10% of simula-

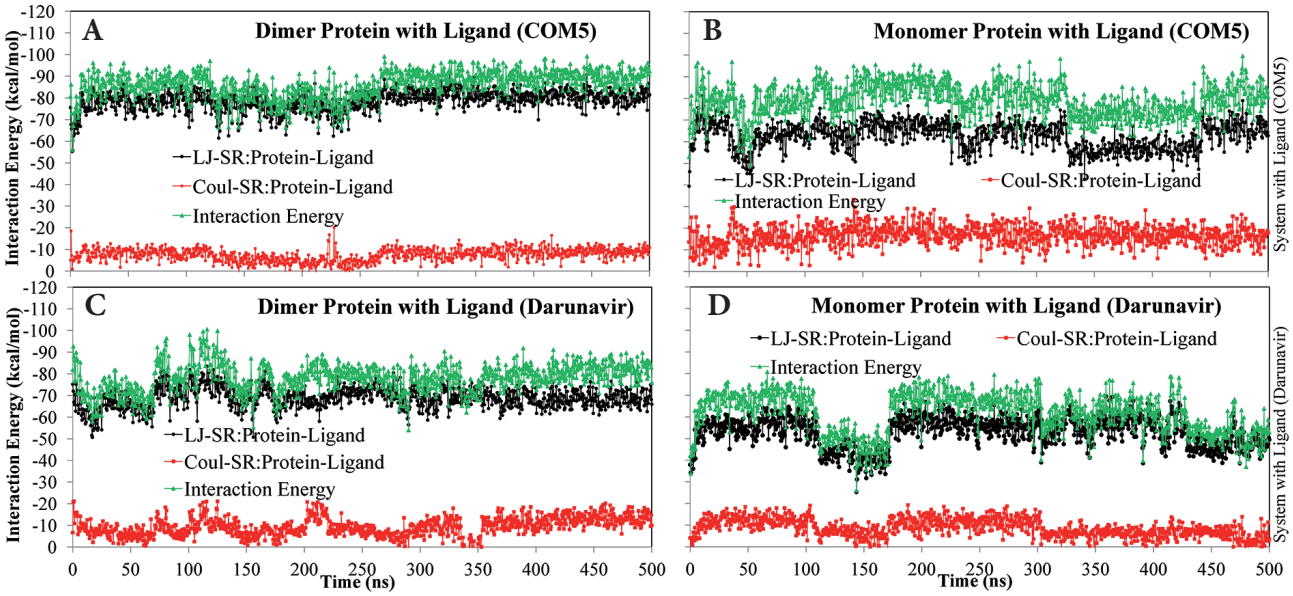

Figure 5. Energies obtained from gromacs simulation for HIV-1 protease with ligands (COM5 or Darunavir). **A.,B.** COM5 interaction energies with monomer and dimer form of HIV-1 protease. **C.,D.** Darunavir interaction energies with monomer and dimer form of HIV-1 protease enzyme.

tion time. HIV-1 protease apo dimer system (without ligand) formed 73 protein-protein H-Bond interaction from which 17 hydrogen bond interactions lasted ≥ 10% of simulation time. Looking the occupancies of each different dimer systems, it was observed that apo (without ligand) dimer system has formed more protein-protein interaction as compared to systems with ligands (COM5 of Darunavir). Average number of protein-protein hydrogen bond for complex HIV-1 protease/COM5 over 500 ns of simulation time were 8 H-Bonds, for HIV-1 protease/Darunavir over 500 ns of simulation time were 9 H-Bonds, and for HIV-1 protease apo form (without ligand) over 1000 ns of simulation time were 12 H-Bonds (Fig. 6). Decrease in number of hydrogen bonds in ligand-bound systems, indicates residues involvement in intermolecular interactions in protein-ligand complexes (Table 1 and S1).

Analyzing intramolecular interaction of HIV-1 protease in complex with and without ligands, it was observed that apo form had formed more H-Bonds as compared to that of systems with ligand (Fig. 6). Monomer form (Chain A) had formed more intramolecular interactions in all three systems as compared to that of monomers from dimer form, which indicates involvement of residues in interaction with second monomer in dimer system (Fig. 6). Similarly in protein-water interactions, it was observed that apo form had formed more H-Bonds with water molecular as compared to that of systems with ligand (Fig. S6).

Dynamics of compounds towards HIV-1 protease

Distance between centre of mass

Distance between centre of masses of ligand-protein and protein-protein were traced during the entire MD simulation time to analyze binding cleft of ligands in detail with HIV-1 protease (Fig. 7). Average distance between the centre of mass between two monomers of dimer system was ~21–22 Å, from which apo form (without ligand) showed steady distance throughout 1000 ns of simulation time as compared to that of both dimer systems with ligand. HIV-1 protease system with Darunavir has shown more jumps in distance and till the end of simulation, distance was almost stable. Analyzing distance of centre of masses between ligand and protein, it was observed that more shift in the distance of ligand with monomer of dimer system, favored in forming more hydrogen bond interactions of ligand with HIV-1 protease (Fig. 7 and Table 1). Average distance between centre of mass of ligand COM5 with Chain A of dimer systems was 16 Å and Chain B of dimer system was 11 Å. For ligand Darunavir average distance between centre of mass was 13 Å with Chain A and 15 Å was with Chain B of dimer system. Average distance between centre of masses of ligand COM5 and Darunavir with monomer systems of HIV-1 protease was 12 Å (Fig. 7).

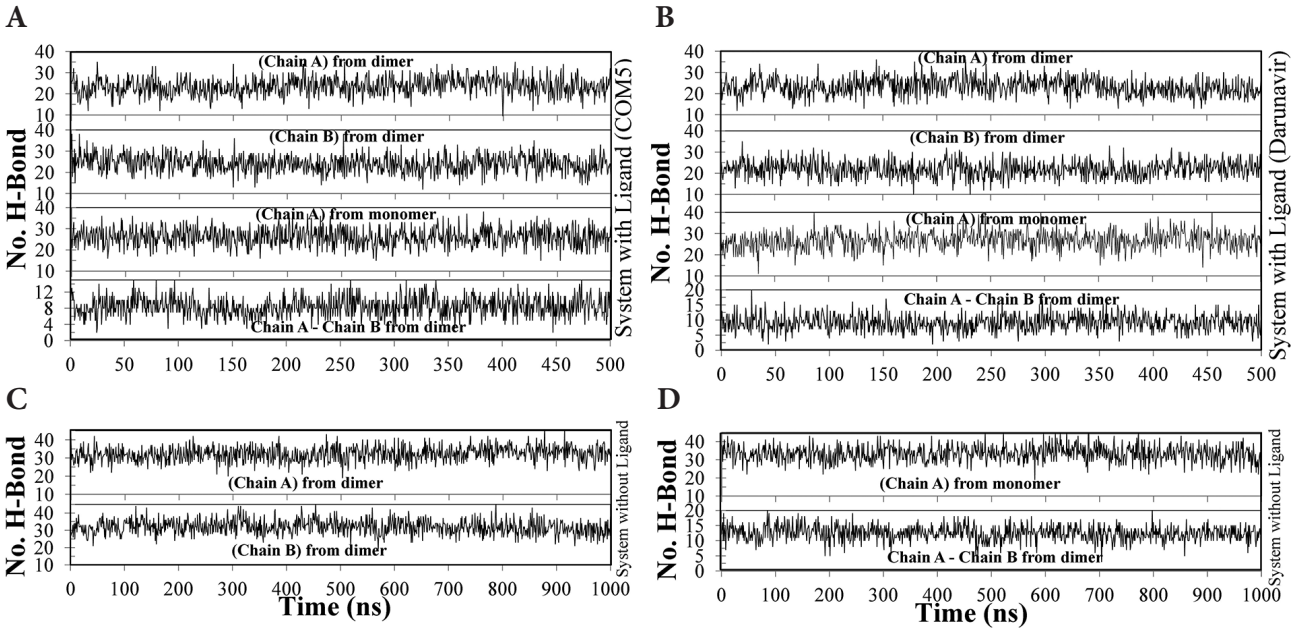

Figure 6. Hydrogen bond interactions of HIV-1 protease intermolecular as well as intramolecular. **A.** Interactions of dimer/monomer of protein in complex with ligand COM5. **B.** Dimer/monomer form of protein interactions from ligand Darunavir complex. **C.** Intramolecular interactions of dimer from of apo HIV-1 protease protein (without ligand). **D.** Intramolecular interactions of monomer from of apo HIV-1 protease protein (without ligand), and intermolecular interactions of two monomers in dimer system).

Flaps open and close behaviour traced during MD

To determine the behaviour of flap region of HIV-1 protease enzyme in ligand-bound and unbound state, we measured distance between ILE50 residues of each monomer from dimer systems throughout molecular dynamic simulation time. Analyzing results it was observed that flap region remained almost closed during entire 500 ns of simulation time with an average distance of ~6 Å between ILE50 residues of flap region from each monomer (Fig. 8) in ligand bound system. A slight fluctuation in the distance value was observed for system bound with ligand Darunavir in beginning of simulation, but after 80 ns it remained steady till end of simulation. For dimer system without ligand, it was observed that distance between ILE50 residues had high fluctuation in the plot ranging from of ~6 Å to of ~14 Å throughout the 1000 ns of simulation time. Results obtained from our molecular dynamic simulation correlates with the mechanism of flap semi-open, open, and close present in work by Viktor et al. (2006). In our work we were able to trace continuous motion of opening and closing (clapping) of flap region in entire 1000 ns simulation time in dimer systems without ligand (Fig. 8).

Discussion

In our current work, we made effort to understand dynamics behaviour of our designed compound COM5 (Kalathiya and Padariya 2014) and its inhibitory activities towards HIV-1 protease enzyme in dimer and monomer form using MD approach. To make comparative analysis, an FDA approved HIV-1 protease inhibitor Darunavir (MacArthur 2007; Kožíšek et al. 2014) was tested with the same methodology. Additionally, an apo form of HIV-1 protease as monomer and dimer form was also studied in order to study response of protein to the ligand. Analysis of results was performed answering six questions raised while initiating MD study. Results from MD suggested that HIV-1 protease behaves different in ligands bound and apo systems, dimer system in apo form was more stable as compared to that of systems with ligand (COM5 or Darunavir). Inverse behavior than dimer was observed in monomer form of HIV-1 protease (apo form), suggesting that ligand activity in dimer systems hinder the stability of HIV-1 protease enzyme.

Our MD results correlate well with work published by Yaakov and Amedeo (2003) and Viktor et al. (2006). Additionally, we were able to trace that flaps behaves slightly different in dimer and monomer form as described in work by Yaakov and Amedeo (2003) and Viktor et al. (2006). From RMSF plots obtained for HIV-1 protease in 500 ns (of ligand bound) and 1000 ns (of apo form) of simulation time, it was observed that flaps fluctuates more in monomer system as compared to dimer where as in work by Yaakov and Amedeo (2003), flap region from both apo systems (monomer and dimer) behaved similar. And in work by Viktor et al. (2006), they observed that for flap region in free state, RMSF value reaching up to 4 Å where as in our work of 1000 ns of MD simulation for apo form, RMSF of flap region reached up to 2 Å. Furthermore, our results correlates with the mechanism of flap semi-open, open, and close as present in work by Viktor et al. (2006), in our work we were able to trace continuous motion of opening and closing (clapping) of flap region during entire 1000 ns simulation (Fig. 8).

Dynamic studies revealed that one monomer from dimer systems is dominant on other monomer in terms of interac-

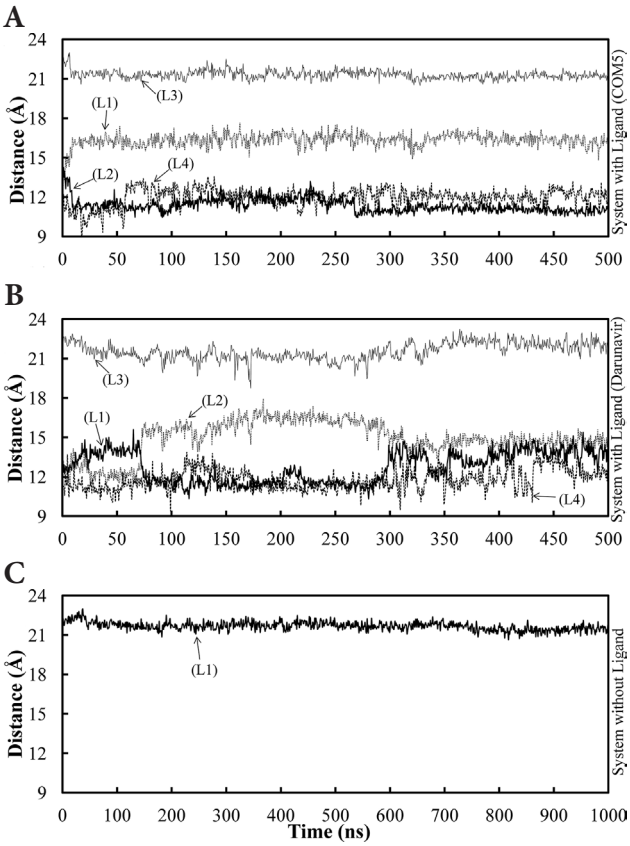

Figure 7. Distance between centre of masses of ligand-protein and protein-protein. **A.** Distance centre of masses of dimer/monomer form of HIV-1 protease protein and ligand COM5. (Liner numbering (L1): Ligand (COM5) – Chain A (Dimer), (L2): Ligand (COM5) – Chain B (Dimer), (L3): Chain A – Chain B (Dimer), and (L4): Ligand (COM5) – Chain A (Monomer)). **B.** Distance centre of masses of dimer/monomer form of HIV-1 protease and Darunavir. (Liner numbering (L1): Ligand (Darunavir) – Chain A (Dimer), (L2): Ligand (Darunavir) – Chain B (Dimer), (L3): Chain A – Chain B (Dimer), and (L4): Ligand (Darunavir) – Chain A (Monomer)) **C.** Distance centre of masses between two monomers of dimer system of HIV-1 protease without ligand. (Liner numbering (L1): Chain A – Chain B (Dimer)).

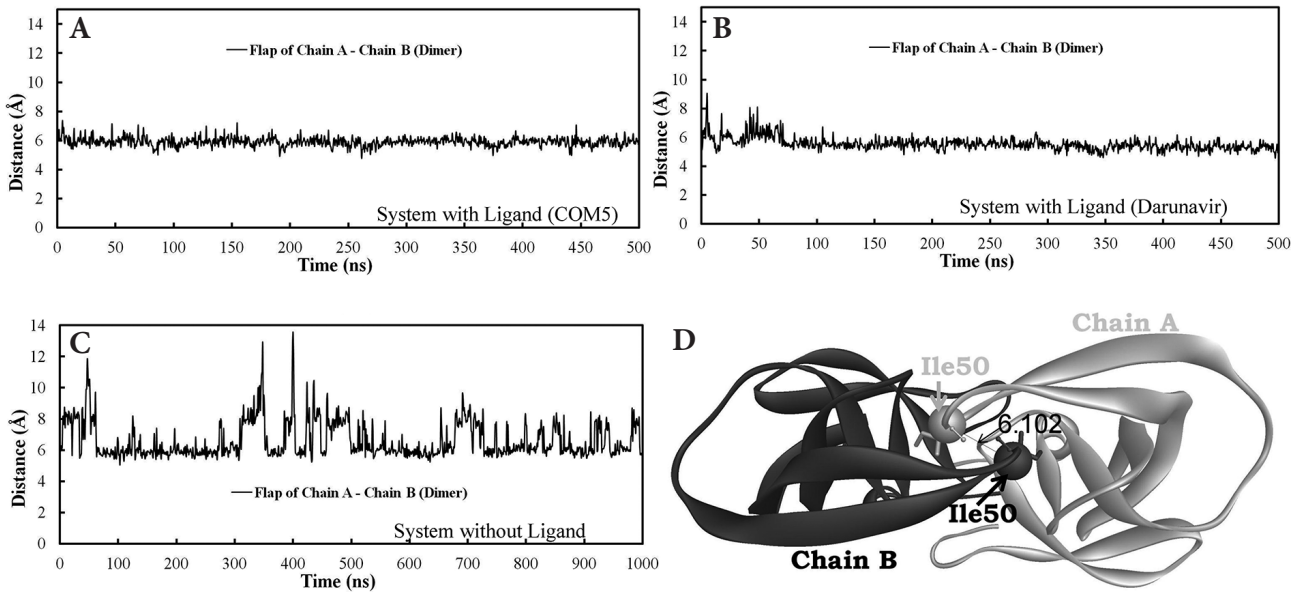

Figure 8. A.,B.,C. Distance between CA atoms of ILE50 of flap region from both monomer of dimer system. **D.** Representation of CA atoms of ILE50 residue of flap region from both monomers of dimer protein and distance between two CA atoms of ILE50.

tion made with ligands. In HIV-1 protease/COM5 complex, Chain A has formed more stable H-Bond with ligand as compared to Chain B where as in HIV-1 protease/Darunavir complex, inverse behavior was observed for Chain A and Chain B (Table 1) and these correlate with the fluctuation in RMSD and RMSF plots of each monomer in ligand bound dimer systems (Fig. 2 and 3). Residues VAL32, ILE50, PRO81, and VAL82 from either of monomer Chain A or Chain B from dimer systems were found to be common forming H-Bond interactions with ligand COM5 or Darunavir. ASP29, ASP30, GLY48, ILE50, and ILE84 residues were commonly involved in H-Bond interaction in monomer systems of HIV-1 protease/COM5 and HIV-1 protease/Darunavir complex, respectively. ASP25, ASP29, ASP30, GLY48, and ILE50 either from dimer or monomer system of HIV-1 protease/COM5 or Darunavir complex, showed similar H-Bond interactions with literature data (Kožíšek et al. 2014; Kalathiya and Padariya 2014; Parrinello and Rahman 1981; van Gunsteren and Berendsen 1988; Bjelkmar et al. 2010; Yaakov and Amedeo 2003). Crude interaction energy was calculated from MD trajectories and comparing interaction energy for ligand COM5 with Darunavir, it was observed that ligand COM5 has more average interaction and stable energies as compared Darunavir (Fig. 5 and Table 2). Average interaction energy for compound COM5/Darunavir with dimer system was –86.03/–78.03 kcal/mol and for monomer system was –79.11/–62.48 kcal/mol.

Concluding, our MD studies emphasized molecular details and conformational changes of HIV-1 protease in ligand bound and unbound state as dimer and monomer form. Our MD results summarize that ligand COM5 designed in our previous studies (Kalathiya and Padariya 2014) shows almost similar behaviour towards HIV-1 protease enzyme as Darunavir (MacArthur 2007; Kožíšek et al. 2014), and propose as promising lead compound for the development of new inhibitor for HIV-1 protease.

Acknowledgement. Academic Computer Center (PLGrid Infrastructure) – Cyfronet, Krakow, Poland provided their hardware and software resources on which molecular dynamics calculations were performed, authors are thankful to them.

References

Ahmed S. M., Maguire G. E., Kruger H. G., Govender T. (2014): The impact of active site mutations of South African HIV PR on drug resistance: Insight from molecular dynamics simulations, binding free energy and per-residue footprints. Chem. Biol. Drug. Des. **83,** 472–481 https://doi.org/10.1111/cbdd.12262

Ali A., Bandaranayake R. M., Cai Y., King N. M., Kolli M., Mittal S., Murzycki J. F., Nalam M. N., Nalivaika E. A., Ozen A., Moses M. P., Kelly T., Celia A. S. (2010): Molecular basis for drug resistance in HIV-1 protease. Viruses **2,** 2509–2535 https://doi.org/10.3390/v2112509

Berman H. M., Westbrook J., Feng Z., Gilliland G., Bhat T. N., Weissig H., Shindyalov I. N., Bourne P. E. (2000): The Protein Data Bank. Nucleic Acids Res. **28,** 235–242 https://doi.org/10.1093/nar/28.1.235

Bjelkmar P., Larsson P., Cuendet M., Hess B., Lindahl E. (2010): Implementation of the CHARMM force field in GROMACS:

analysis of protein stability effects from correction maps, virtual interaction sites, and water models. J. Chem. Theory Comput. **6,** 459–466
https://doi.org/10.1021/ct900549r

Brower E. T., Bacha U. M., Kawasaki Y., Freire E. (2008): Inhibition of HIV-2 protease by HIV-1 protease inhibitors in clinical use. Chem. Biol. Drug Des. **71,** 298–305
https://doi.org/10.1111/j.1747-0285.2008.00647.x

Bussi G., Donadio D., Parrinello M. (2007): Canonical sampling through velocity rescaling. J. Chem. Phys. **126,** 014101
https://doi.org/10.1063/1.2408420

Chapman T. M., Plosker G. L., Perry C. M. (2004): Fosamprenavir – a review of its use in the management of antiretroviral therapy-naive patients with HIV infection. Drugs **64,** 2101–2124
https://doi.org/10.2165/00003495-200464180-00014

Darden T., York D., Pedersen L. (1993): Particle mesh Ewald: An Nlog(N) method for Ewald sums in large systems. J. Chem. Phys. **98,** 10089–10092
https://doi.org/10.1063/1.464397

Davies D. R. (1990): The structure and function of the aspartic proteinases. Annu. Rev. Biophys. Biophys. Chem. **19,** 189–215
https://doi.org/10.1146/annurev.bb.19.060190.001201

Dierynck I., Keuleers I., deWit M., Tahri A., Surleraux D. L., Peeters A., Hertogs K. (2005): Kinetic characterization of the potent activity of TMC114 on wild-type HIV-1 protease. (Poster 64), 14th International HIV Drug Resistance Workshop, Québec City, Canada

Elio A. C., Wing-Yiu C., Mikko K. (2012): Comparison of secondary structure formation using 10 different force fields in microsecond molecular dynamics simulations. J. Chem. Theory Comput. **8,** 2725–2740
https://doi.org/10.1021/ct300323g

Eron J. J. (2000): HIV-1 protease inhibitors. Clin. Infect. Dis. **30,** 160–1670
https://doi.org/10.1086/313853

Flexner C. (1998): HIV-protease inhibitors. N. Engl. J. Med. **338,** 1281–1292
https://doi.org/10.1056/NEJM199804303381808

Flexner C. (2007): HIV drug development: the next 25 years. Nat. Rev. Drug. Discov. **6,** 959–966
https://doi.org/10.1038/nrd2336

Ghosh A. K., Dawson Z. L., Mitsuy H. (2007): Darunavir, a conceptually new HIV-1 protease inhibitor for the treatment of drug-resistant HIV. Bioorg. Med. Chem. **15,** 7576–7580
https://doi.org/10.1016/j.bmc.2007.09.010

Grant S. K., Deckman I. C., Culp J. S., Minnich M. D., Brooks I. S., Hensley P., Christine D., Thomas D. M. (1992): Use of protein folding studies to determine the conformational and dimeric stabilities of HIV-1 and SIV proteases. Biochemistry **31,** 9491–9501
https://doi.org/10.1021/bi00154a023

Hess B., Bekker H., Berendsen H. J., Fraaije J. G. (1997): LINCS: A linear constraint solver for molecular simulations. J. Comput. Chem. **18,** 1463–1472
https://doi.org/10.1002/(SICI)1096-987X(199709)18:12<1463::AID-JCC4>3.0.CO;2-H

Hess B., Kutzner C., van der Spoel D., Lindahl E. (2008): GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J. Chem. Theory Comput. **4,** 435–447
https://doi.org/10.1021/ct700301q

James M. N., Sielcki A. (1983): Structure and Refinement of penicillopepsin at 1.8 A resolution. J. Mol. Biol. **163,** 299–361
https://doi.org/10.1016/0022-2836(83)90008-6

Jaskólski M., Tomasselli A. G., Sawyer T. K., Staples D. G., Heinrikson R. L., Schneider J., Kent S. B., Wlodawer A. (1991): Structure at 2.5-A resolution of chemically synthesized human immunodeficiency virus type 1 protease complexed with a hydroxyethylene-based inhibitor. Biochem. **30,** 1600–1609
https://doi.org/10.1021/bi00220a023

Kalathiya U., Padariya M. (2014): Inhibiting activity of HIV-1: protease, reverse transcriptase and integrase all together by novel compounds using computational approaches. Int. J. Biosci. Biochem. Bioinforma. **4,** 448–457
https://doi.org/10.17706/ijbbb.2014.4.6.448-457

Katoh I., Yasunaga T., Ikawa Y., Yoshinaka Y. (1987): Inhibition of retroviral protease activity by an aspartyl proteinase inhibitor. Nature **329,** 654–656
https://doi.org/10.1038/329654a0

King N. M., Prabu-Jeyabalan M., Nalivaika E. A., Wigerinck P., deBethune M. P., Schiffer C. A. (2004): Structural and thermodynamic basis for the binding of TMC114, a next-generation human immunodeficiency virus type 1 protease inhibitor. J. Virol. **78,** 12012–12021
https://doi.org/10.1128/JVI.78.21.12012-12021.2004

Kohl N. E., Emmi E. A., Schleif W. A., Davies L. J., Heimbach J. C., Dixon R. A. (1988): Active human immuno-deciency virus protease was required for viral infectivity. Proc. Natl. Acad. Sci. **85,** 686–690
https://doi.org/10.1073/pnas.85.13.4686

Kožíšek M., Lepšík M., Grantz Šašková K., Brynda J., Konvalinka J., Rezáčová P. (2014): Thermodynamic and structural analysis of HIV protease resistance to Darunavir - analysis of heavily mutated patient-derived HIV-1 proteases. FEBS J. **281,** 1834–1847
https://doi.org/10.1111/febs.12743

Lascar R. M., Benn P. (2009): Role of Darunavir in the management of HIV infection. HIV AIDS (Auckl) **1,** 31–39
https://doi.org/10.2147/hiv.s5397

Lefebvre E., Schiffer C. A. (2008): Resilience to resistance of HIV-1 protease inhibitors: profile of Darunavir. AIDS Rev. **10,** 131–142

Leonis G., Czyżnikowska Ż., Megariotis G., Reis H., Papadopoulos M. G. (2012): Computational studies of Darunavir into HIV-1 protease and DMPC bilayer: necessary conditions for effective binding and the role of the flaps. J. Chem. Inf. Model. **52,** 1542–1558
https://doi.org/10.1021/ci300014z

Levy Y., Caflisch A., Onuchic J. N., Wolynes P. G. (2004): The folding and dimerization of HIV-1 protease: evidence for a stable monomer from simulations. J. Mol. Biol. **340,** 67–79
https://doi.org/10.1016/j.jmb.2004.04.028

Lindahl E., Hess B., van der Spoel D. (2001): GROMACS 3.0: a package for molecular simulation and trajectory analysis. J. Mol. Model. **7,** 306–317
https://doi.org/10.1007/s008940100045

Luber A. D., Brower R., Kim D., Silverman R., Peloquin C. A., Frank I. (2007): Steady-state pharmacokinetics of once-daily fosamprenavir/ritonavir and atazanavir/ritonavir alone and in combination with 20 mg omeprazole in healthy volunteers. HIV. Med. **8,** 457–464 https://doi.org/10.1111/j.1468-1293.2007.00496.x

MacArthur R. D. (2007): Darunavir: promising initial results. Lancet **369,** 1143–1144 https://doi.org/10.1016/S0140-6736(07)60499-1

Madruga J. V., Berger D., McMurchie M., Suter F., Banhegyi D., Ruxrungtham K., Norris D., Lefebvre E., deBthune M. P., Tomaka F., Pauw M. D., Vangeneugden T., Spinosa-Guzman S. (2007): Efficacy and safety of Darunavir-ritonavir compared with that of lopinavir-ritonavir at 48 weeks in treatment-experienced, HIV-infected patients in titan: a randomised controlled phase III trial. Lancet **370,** 49–58 https://doi.org/10.1016/S0140-6736(07)61049-6

McCoy C. (2007): Darunavir: A nonpeptidic antiretroviral protease inhibitor. Clin. Therapeut. **29,** 1559–1576 https://doi.org/10.1016/j.clinthera.2007.08.016

Miller M., Schneider J., Sathyanarayana B. K., Toth M. V., Marshall G. R., Clawson L., Selk L., Kent S. B., Wlodawer A. (1989): Structure of complex of synthetic HIV-1 protease with a substrate-based inhibitor at 2.3 A resolution. Science **246,** 1149–1152 https://doi.org/10.1126/science.2686029

Moonsamy S., Soliman M. E. (2013): Dual acting HIV inhibitors: integrated rational in silico design strategy. Med. Chem. Res. **23,** 682–689 https://doi.org/10.1007/s00044-013-0670-9

Moore J. P., Stevenson M. (2000): New targets for inhibitors of HIV-1 replication. Nat. Rev. Mol. Cell. Biol. **1,** 40–49 https://doi.org/10.1038/35036060

Palella F. J., Delaney K. M., Moorman A. C., Loveless M. O., Fuhrer J., Satten G. A., Aschman D. J., Holmberg S. D. (1998): Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N. Engl. J. Med. **338,** 853–860 https://doi.org/10.1056/nejm199803263381301

Parrinello M., Rahman A. (1981): Polymorphic transitions in single crystals: A new molecular dynamics method. J. Appl. Phys. **52,** 7182–7190 https://doi.org/10.1063/1.328693

Perryman A. L., Lin J. H., McCammon J. A. (2004): HIV-1 protease molecular dynamics of a wild-type and of the V82F/I84V mutant: possible contributions to drug resistance and a potential new target site for drugs. Protein Sci. **13,** 1108–1123 https://doi.org/10.1110/ps.03468904

Qiu X., Zhao G. D., Tang L. Q., Liu Z. P. (2014): Design and synthesis of highly potent HIV-1 protease inhibitors with novel isosorbide-derived P2 ligands. Bioorg. Med. Chem. Lett. **24,** 2465–2468 https://doi.org/10.1016/j.bmcl.2014.04.008

Rang H. P., Dale M. M., Ritter J. M., Flower R. J. (2007): Rang and Dale's Pharmacology. (6th edition). Philadelphia Churchill Livingstone Elsevier

Schobert R., Stehle R., Walter H. (2008): Tipranavir analogous 3-sulfonylanilidotetronic acids: new synthesis and structure-dependent anti-HIV activity. Tetrahedron **64,** 9401–9407 https://doi.org/10.1016/j.tet.2008.07.094

Schuler L. D., Daura X., VanGunsteren W. F. (2001): An improved GROMOS96 force field for aliphatic hydrocarbons in the condensed phase. J. Comput. Chem. **22,** 1205–1218 https://doi.org/10.1002/jcc.1078

Todd M. J., Semo N., Freire E. (1998): The structural stability of the HIV-1 protease. J. Mol. Biol. **283,** 475–488 https://doi.org/10.1006/jmbi.1998.2090

UNAIDS Global report (2013): UNAIDS report on the global AIDS epidemic. Joint United Nations Program on HIV/AIDS (UNAIDS)

van Gunsteren W. F., Berendsen H. C. (1988): A leap-frog algorithm for stochastic dynamics. Mol. Simul. **1,** 173–185 https://doi.org/10.1080/08927028808080941

Viktor H., Asim O., Robert C. R., Carlos S. (2006): HIV-1 protease flaps spontaneously open and reclose in molecular dynamics simulations. Proc. Natl. Acad. Sci. USA **103,** 915–920 https://doi.org/10.1073/pnas.0508452103

Wartha F., Horn A. H., Meiselbach H., Sticht H. (2005): Molecular Dynamics Simulations of HIV-1 Protease Suggest Different Mechanisms Contributing to Drug Resistance. J. Chem. Theory. Comput. **1,** 315–324 https://doi.org/10.1021/ct049869o

Weber I. T., Agniswamy J. (2009): HIV-1 protease: structural perspectives on drug resistance. Vir. **1,** 1110–1136 https://doi.org/10.3390/v1031110

WHO (2015): 19th WHO Model List of Essential Medicines

Wlodawer A., Vondrasek J. (1998): Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. Annu. Rev. Biophys. Biomol. Struct. **27,** 249–284 https://doi.org/10.1146/annurev.biophys.27.1.249

Wlodawer A. (2002): Rational approach to AIDS drug design through structural biology. Annu. Rev. Med. **53,** 595–614 https://doi.org/10.1146/annurev.med.53.052901.131947

Xie D., Gulnik S., Gusrchina E., Yu B., Shao W., Qoronfleh W., Anand N., John W. E. (1999): Drug resistance mutations can affect dimer stability of HIV-1 protease at neutral pH. Protein Sci. **8,** 1702–1707 https://doi.org/10.1110/ps.8.8.1702

Yaakov L., Amedeo C. (2003): Flexibility of monomeric and dimeric HIV-1 protease. J. Phys. Chem. B. **107,** 3068–3079 https://doi.org/10.1021/jp0219956

Yanchunas J., Langley D. R., Tao L., Rose R. E., Friborg J., Colonno R. J., Doyle M. L. (2005): Molecular basis for increased susceptibility of isolates with atazanavir resistance-conferring substitution I50L to other protease inhibitors. Antimicrob. Agents Chemother. **49,** 3825–3832 https://doi.org/10.1128/AAC.49.9.3825-3832.2005

Received: April 13, 2016
Final version accepted: June 24, 2016
First published online: December 6, 2016