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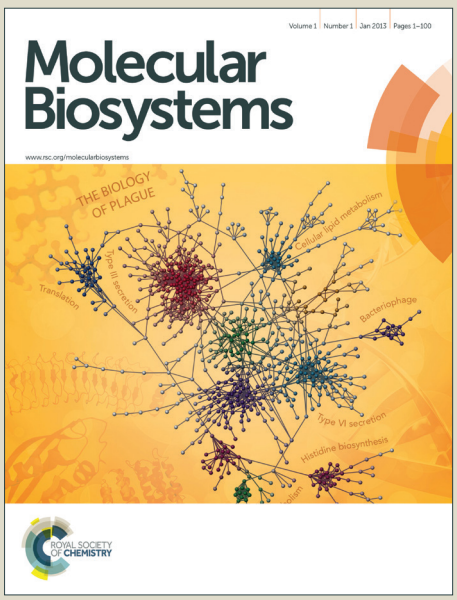

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Molecular insights of protein contours recognition with ligand pharmacophoric sites through combinatorial library design and MD simulation in validating HTLV-1 PR inhibitors

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Short title: ***Ligand Pharmacophore recognition in Protein contours***

Molecular insights of protein contours recognition with ligand pharmacophoric sites through combinatorial library design and MD simulation in validating HTLV-1 PR inhibitors

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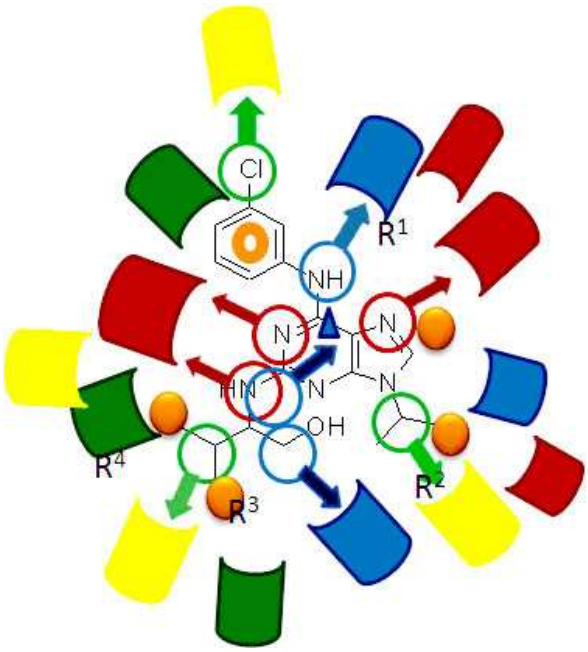

Binding interactions are analyzed through charge environment present in both ligand pharmacophoric site and protein active site. Domino effect illustrates those libraries of Purvalanol-A, are attuned to fill allosteric binding site of HTLV-1 PR through molecular recognition and shows proper binding of ligand Pharmacophoric features in receptor contours.

Abstract

Retroviruses HIV-1 and HTLV-1 are chiefly considered as most dangerous pathogens in Homo sapiens. These two viruses are having structurally unique protease enzyme, which are common for its replication mechanism. Though HIV PR drugs were failed to inhibit the HTLV-1 infections, it emphatically insists the need for designing a new lead compounds against HTLV-1 PR. Therefore, we tried to understand the binding level interactions through charge environment present in both ligand and protein active site. While domino effect illustrates that libraries of Purvalanol-A, are attuned to fill allosteric binding site of HTLV-1 PR through molecular recognition, and shows proper binding of ligand Pharmacophoric features in receptor contours. Our screening, evaluates seven compounds from purvalanol-A libraries, and these compounds pharmacophore searches appropriate place in binding site and it places well according to respective receptor contour surfaces. Thus our result provides a platform for the progress of more effective compounds, which are better in Free energy calculation, molecular docking, ADME and molecular dynamics studies. Finally, this research provided novel chemical scaffolds for HTLV-1 drug discovery.

Key words: *Active site; Binding energy; Contour map; HTLV; Molecular recognition; pharmacophore*

Introduction

The retrovirus human T-cell lymphotropic virus type 1 (HTLV-1) was discovered in the early 1980s. HTLV-1 infection associated to the development of adult T-cell leukemia/ lymphoma (ATL/ATLL), a clonal aggressive malignancy of $CD4^+$ T lymphocytes[1-2]. At present, HTLV-1 infects approximately 20 million individuals all around the world and it is the first retrovirus to be unambiguously linked causally to a human cancer[3].Resembling with human immunodeficiency virus (HIV), HTLV-1 mainly infects CD4 T-cells, which are the central regulators of the acquired immune response[4]. To establish persistent infection, HTLV-1 perturbs the regulation of CD4 T cells, sometimes which leads to adult T-cell leukemia (ATL) [5-6]. When compare to other retroviruses, HTLV-1 encodes protease (PR) enzyme is essential for viral maturation [7]. Therefore on targeting protease enzyme in HTLV-1 PR prevents viral proliferation and maturation, which makes PR enzyme as key drug target for the development of new potential lead compounds [8]. Tozer *et al.,* (2007) stated that, although the mechanism by which the virus causes diseases is still not known, but their studies indicate that viral replication is critical for the development of HTLV-1 associated myelopathy, and initial studies suggested that blocking replication with PR inhibitors had a therapeutic effect[9]. Therefore, based on the success of HIV-1 PR inhibitors, the HTLV-1 PR is also considered as a potential target for chemotherapy [10]. But recent research reports, states that anti HIV PR drugs cannot function as HTLV-1 PR blockers and several successful HIV-1 PR inhibitors failed to provide the inhibitory activity against HTLV-1 PR [8, 11], so prediction of a potent inhibitor for HTLV-1 PR is highly essential for human welfare.

Li *et al.,* (2005) has crystallographically solved the X-ray structure of a HTLV-1 PR and reported that, HTLV-1 PR enzyme is an attractive drug target for anti-cancer drug design [12].

Structure of HTLV-1 PR represents the homodimer, contains 125 residues per chain, which are longer sequence compared to HIV-1 PR [13].

The two chains of HTLV-1 PR monomer are bound by non-bonded interactions with active site, at the interface between two monomers [14]. Whereas in similar to HIV PR, the HTLV-1 PR also shows same position of active site between the two monomeric chains, but HTLV-1 protease is elongated by an extra 10 amino acid residue “tail” at the C-terminus [15]. Therefore the active site region is expanded in HTLV-1 PR, and the known HIV PR drugs cannot sustain the inhibitory profile against the HTLV-1 PR [16]. While the structural information of HTLV-1 PR complexed with inhibitor (PDB id 3LIN, 3LIQ, 3LIT, 3LIV, 3LIX and 3LIY), shows that active sites are located between the two monomer chains comprising of ARG10, LEU30, ASP32, GLY34, ALA35, ASP36, MET37, VAL39, LEU56, LEU57, ALA59, LEU91, TRP98 and LLE100 amino acids These residues are known to contribute to the binding interactions and have the capacity to accommodate ligand and inhibit the drug target [12].

The presence of active site information boost up the structure based drug discovery to design HTLV-1 PR inhibitors, while improved understanding of active site contour features is an important reference for pocket specific *De novo* chemical library screening and scoring functions [17]. Combine approach of receptor environment and screening of libraries with protein−ligand charge distribution was computed by the scoring function to recognize the importance of pharmacophoric features, which elucidates the arrangement of chemical features that are shared by molecules exhibiting activity at receptor [18-20]. Currently, predicting accurate binding free energies of new leads in particular receptor site is a challenging one and from this work, we have consistently introduced a numbers of structural adaptation strategies in the original structure of Purvalanol-A, which induce viral proliferation and

apoptosis [21-22]. Here, charge calculation of receptor active site for predicting chemical requirement (Contour maps) were carried out using SiteMap, which have the potential to accept the ligand with respective pharmacophoric features (chemical sharing of receptor-ligand). Thus the contour maps based interaction analysis between ligand and protein is crucial for developing new inhibitors. The protein contour maps are charged environments which interact only with their respective pharmacophoric site of small molecules [23-25]. The hydrogen bond acceptor, hydrogen bond donor, hydrophobic group, negatively charged group, positively charged group, aromatic ring pharmacophoric features in ligands tend to bind with structural factors of HTLV-1 PR surface of active site macromolecule was shown in **figure 1 and Figure S1 (a and b).** Although contour based lead optimization enhance the vitality of understanding to find out effective and potent drug for HTLV-1 PR [25-27].

Materials and Methods

System Configuration

All studies were carried out in a high-performance cluster operated with Cent OS V6.2 Linux operating platform. The hardware specifications are HPC cluster -Super micro SC826TQ-R1200 LIB series, running with two Intel Xeon E5620 Quad Core 2.4-GHz processors on 32-GB RAM. The software specifications included are the academic version of Gromacs v4.5 for MD simulations and the commercial version of Schrodinger 2012 software package (Version 9.2; LLC, New York, NY) for docking protocol.

Molecular Modeling environmental Setup

The typical structure file from the PDB is not suitable for immediate use in molecular modeling calculations and so the crystal structure of HTLV-1 PR (PDB id: 2B7F) is prepared through protein preparation wizard[12]. The two monomers chain A and B were taken for

subsequent investigation. By using the Prime, missing residues and the missing loops are filled from the SEQRES records in the PDB file. Firstly, the bond orders were assigned, hydrogen atoms were added and all the crystallographic waters without any contact were removed. Minimization was performed until the average RMSD of the non-hydrogen atoms to reach 0.3Å. The default sampling and water optimization using OPLS-2005 force field and impref minimization were applied to prepare the apo protein. The reported inhibitor of HTLV-1 PR, namely Purvalanol-A, was prepared with Lig-Prep 2.4 using OPLS-2005 force field. The preparation was done so as to retain original state of ligand and chirality. Up to 32 conformations per ligand structure were generated using the default energy ring confirmation [28].

Binding site and contour map prediction

Druggability sites are located through clustering the favorable regions by means of vdW charges on protein surface using Sitemap 2.4 [23]. It implies OPLS-2005 force field parameters to estimate the interaction energies of probes placed at all points on three dimensional grids that encompass the entire protein. To identify the top ranked potential druggability sites, it requires at least 10 site points, and then the environment is set more restrictive by definitions of hydrophobicity, using standardized grid and crop site maps at 3Å[29]. The requirement of physico-chemical properties in ligands to design best inhibitor against the protein active site contours are predicted by using sitemap[30]. The docked results of known inhibitors are subjected to physico-chemical properties of protein contours prediction. Ligand molecule was then picked manually to evaluate the single binding region around the inhibitor and additional region around 6Å buffer for examination [23].

Combinatorial Library design

Based on protein physio-chemical requirement on active site, the rearrangement regions are noted in the Purvalanol-A structure and by using the ligand designer script R^1, R^2, R^3 and R^4 sites are picked (**Figure S1 (a and b))** [31]**.** Addition of atoms (Chain structure, Cycle structure, C-groups, Miscellaneous, Protecting groups details are given in supplementary information **figure S2**) from ChemSketch program [32] and of radicals in each R-position is made and imported in Maestro and optimized through OPLS-2005 force field using ligprep [28.]

Molecular docking Simulation

The interactions between the protein and ligand are carried out by the molecular docking program Glide V.6.1 [33]. Here two different type of docking methodology are performed. While for Purvalanol-A, experimental active site based docking is performed and for the designed inhibitors druggability site based docking is performed [34-37].

Receptor information based grid generation

For Purvalanol-A, the receptor grid generation is processed manually by picking the ligand entry and specifying the centroid of specific residues including ARG10, LEU30, ASP32, GLY34, ALA35, ASP36, MET37, VAL39, LEU56, LEU57, ALA59, LEU91, TRP98, and LLE100 [12]. The position of spherical region that should be occupied by a particular ligand during docking was set as XYZ axis with co-ordinates 92.80, 53.33 and 54.16 respectively. The same active site residues were picked for the HBONDS constraints which are designated as flexible residues [37].

Site based Grid generation

The druggability site based on top ranked Site Score in sitemap was prearranged as Glide input files and the receptor grid generation was carried out with the white colored

spheres along the protein [38]. To recognize the ligand position, the entry of white spheres are picked using the van der Waals radius scaling factor of 1.0 and partial charge cutoff of 0.25. Here the position of grid box is set as XYZ axis with the measurement of 87.94, 55.59, and 54.43 respectively with radius 2.0 [39].

Druggability Site and Receptor based ligand docking

Both Receptor and druggability site based docking was performed with XP docking protocol[33]. Here, Glide generates conformation internally and passes those conformations through a series of filters. In the first stage the ligand center at various grid position of 1.00Å was placed and the ligands were allowed to rotate around the three angles. While at this point of time dissimilar binding modes of the ligands were removed based on the crude score values and geometrical filter. Whereas in the second filter stage a grid-based force field evaluation and refinement of docking solutions including torsional and rigid body movements of the ligand was analyzed through the OPLS-AA force field. A small number of surviving docking solution was subjected to a Monte Carlo procedure for minimized energy score. Subsequently the final energy evaluation was done with the Glide Score and a single best pose was generated as output for a particular ligand with the help of following equation.

Gscore = a*vdW + b *Coul + Lipo +Hbond + Metal + BuryP + RotB + Site

Where vdW = van der Waal energy; Coul = Coulomb energy; Lipo = Lipophilic contact term; HBond = Hydrogen-bonding term; Metal = Metal-binding term; BuryP= Penalty for buried polar group; RotB = Penalty for freezing rotable bonds; Site = Polar interaction at active site; and the coefficient of vdW and Coul are: a = 0.065, b = 0.0130.

The above equation tells about the Gscore which include the main factors of Van der Waals energy, Coulomb energy, hydrophobic grid potential, Hydrogen-bonding term, Metal-

binding term, buried polar groups, freezing rotatable bonds and Polar interactions in the active site [34-35].

Physiochemical descriptors calculation

The eMBrAcE, Prime MM-GBSA, Liaison and Qikprop calculations for the ligand-receptor complex structures were performed to generate the ligand & structure-based descriptors (LSBD) [40]. While eMBrAcE applies multiple minimizations using OPLS-2005 with the surface generalized born implicit water solvent model. Whereas constant dielectric electrostatic treatment were applied specifically 1.0 (default) and non-bonded cutoff a criterion is extended and iterations are counted up to 5000 which calculates molecular mechanics energy minimization of the complex [41-42]. The permeability, adsorption, dissolution, metabolism, and excretion (ADME) properties of compounds were done with Qikprop [43].

Molecular recognition with pharmacophores

Best compounds on docking and free energy analysis are selected to create pharmacophore sites (site points) using PHASE. Phase supplies a built-in set of six pharmacophore features, which includes Hydrogen bond acceptor (A), Hydrogen bond donor (D), Hydrophobic group (H), Negatively charged group (N), Positively charged group (P), Aromatic ring (R) [44-45]. Pharmacophoric features are defined by a set of chemical structure patterns, that having the active part of the drug molecule, particularly structure based target. Based on this pharmacophoric sites, the binding contours of active site is visualized for molecular recognition [46].

Molecular Dynamics Simulation

The MD simulation studies have been carried for the crystal structure of HTLV-1 PR

for 20ns of timescale, in order to understand the stability and intra-molecular conformational changes occurs in the protein. The GROMACS program package (http://www.gromacs.org) adopting the OPLS-AA force field parameters were used for energy minimization and MD simulations [47]. For the MD simulation studies, the structure was solvated using the TIP3P water model, and the solvated structure was energy minimized using steepest descent method, terminating when maximum force is found smaller than 100 KJ mol^{-1} nm^{-1}. The total simulation were performed in the NPT ensemble at constant temperature (300 K) and pressure (1 bar), with a time step of 2fs. While NVT were performed for 1ns, and the minimized structure was equilibrated with a timescale of 20ns [49-50]. Additionally, the MD simulations are performed for the ligand bound docked structures of HTLV-1 PR which are the outcome of combinatorial library design and free energy analysis. The initial structure of the receptor and ligands are cleaned by using GROMOS96 force field and then the topology files were generated for the receptor and ligands separately using PRODRG tool[51]. The simulation system was created manually by importing the ligand topology into the system pursued along with a dodecahedron box with a margin of 1nm and the system was filled with water using the SPC explicit solvation model. While the system was applied with energy minimization and the atomic velocities were adjusted according to Maxwell Boltzmann distribution at 300K with a periodic scaling of 0.1ps. A presimulation run of 20ps was applied to relax the system and to remove the geometric restrains which eventually appeared at the initialization of the run. All the simulations were carried out at constant pressure and temperature (NPT) ensemble. The Berendsen coupling was employed to maintain a constant temperature of 300K and constant semi-isotropic pressure of 1 bar with coupling time of 2.0fs and the coordinates were saved**.** The simulation timescale for ligand bound form is 20ns and

the RMSD analysis has been performed for understanding the stability of ligands [52].

Results and Discussion

Active Site of HTLV-1 PR – Experimental and Theoretical analysis

The comparison of Experimental and Theoretical active site will enhance the binding mode and also to make involvement of few other amino acids as active sites. Where as the information of active site in both experimental (crystallographic information) and theoretical prediction provides strong support for the computational analysis of protein/small molecule interactions. In this study based on the available complex bound HTLV-1 PR crystal structures with Statine inhibitors, we understand that amino acids of ARG10, LEU30, ASP32, GLY34, ALA35, ASP36, MET37, VAL39, LEU56, LEU57, ALA59, LEU91, TRP98 and LLE100 were designated as active sites for the crystal structure of Human T-cell leukemia virus protease (PDB id: 2B7F). Theoretically predicted druggability regions coding amino acids are observed in maestro, shows white colored spheres appeared between two monomer chains, in the Z-Shaped surface (Figure S3). Through Knowledge based method, the druggability sites are noted as ARG10, LEU30, LEU31, ASP32, THR33, GLY34, ALA35, ASP36, MET37, THR38, VAL39, SER 55, LEU56, LEU57, GLY58, ALA59, LEU91, ASN97, TRP98, LLE100. But meanwhile we noticed that, few amino acids in theoretical are not experimentally proven as active sites, and so molecular docking of library compounds may suggest the ability of new active sites. In our previous works, we have mentioned the residue of Met37 has the mutational effect and so, here the purvalanol A has redocked with M37D residue (**Figure S4**). Interestingly, the wild type interaction residues have not taken the interactions role with M37D residue and purvalanol A has lost its ability to bind with HTLV-1PR. The reported compounds

from this work, if it has the ability to bind with Met37 will also have the ability to bind with mutant HTLV-1 PR [53-54].

Purvalanol-A interaction and Generation of contour maps

Purvalanol A to HIV-1 PR has not yet reported, but in case of HTLV-1 it has been reported that, purvalanol A has the effect of inhibition [22]. To understand interaction of the known HTLV-1 PR active Purvalanol-A inhibitor, we have docked Purvalanol-A with known active site residues. It shows very good interactions and bound complex in between both chain A (GLY34) and chain B (ASP32, GLY34) amino acids (Figure 2). These molecular interactions are considered for contour map generation, to check physio-chemical property requirements of receptor active site. Through this contour map analysis, whole active site area of the receptor protein is invigilated and their structural properties are elucidated. Depend on the results, we understand that HTLV-1 PR requires plenty of HPR from the ligand molecules, HBAR, HBDR, HPBR are available in range and absence of MBR in receptor active site. The surface area of each region of contour maps with the respect to Purvalanol-A is reported in Table **1**. In visualization of contour maps, each HPR (green mesh), HBAR (red mesh), HBDR (blue mesh), HPBR (yellow mesh) are shown in figure 3.

Combinatorial library design

The ligand rearrangement position is marked with basement of receptor contours and R^1, R^2, R^3, R^4 position are drawn **(Figure S1)** and based on this, more replacement are done in their R position (Derivatives of Purvalanol-A R_1, R_2, R_3, R_4 are provided in supplementary material **Figure S2**). The glide docking program was employed as primary docking search engine to dock library compounds with that of the predicted druggability regions of HTLV-1 PR. The white colored sphere covers the 4Å area of binding pocket representing a binding

cleft, where the ligand molecule can bind specifically at the region. When ligand molecules replaced the white color dots in the binding cleft region, the atomic structure arrangements attained their electronic level and sharing of hydrogen bonds between the acceptor and donor of the ligands and receptors occurred. Resulting docking studies were evaluated for the binding mechanism of library compounds with HTLV-1 PR and it has the more negative value of G-score which indicated good binding affinity of the ligand with the receptor (Supplementary information Table S1, S2, S3 and S4). On assessment with all library compounds, the best compounds are chosen which comes top in Gscore criteria and forms better interactions. In this obtained conformation of ligands, the numbers of hydrogen bonds formed are noted with respective amino acid involvement.

Rationalization of active sites in ligand recognition

Theoretical active site prediction using sitemap, predicts more correlation in results, with experimental active sites, confirmation of active site are theoretically checked by libraries of Purvalanol-A interaction results with better Gscore than Known Purvalanol-A is considered for active site residue analysis. In a refined docking environment, hydrogen bonding interactions are fully based on donor and acceptors present in the protein and ligands. The atoms in the donor and acceptor regions are playing the charge based interactions, i.e., the positive and negative region between the hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) are able to interact. The atom having the capacity to donate its hydrogen atom towards the acceptor regions to form bonding bridges are called as HBD atoms and the atom having tendency to accept the donated hydrogen atoms are called as HBA atoms. The hydrogen bonding formed in between the HBD and HBA throughout the ligand-receptor interaction was accounted and active sites are analyzed (Table 2). Each

interaction are tabulated and provided in Table **S1, S2, S3, S4** of supplementary information. The libraries of Purvalanol A, which shows better results than source inhibitor in terms of Gscore, are analyzed with active site information for site matching for ligand patterns with reference experimental active site information. Based on Gscore and energy parameters, nine best compounds are chosen, i.e. Fluroenylmethoxycarbonyl (R1 -A), Phosphate (R2 - B), Phosphate (R4 - C), Benzyloxycarbonyl (R4 - D), Phosphate (R2 -E), Sulfate (R3 -F), Phenyloxycarbonyl (R4- G), Trifluroacetyl (R3 -H) and Fluroenylmethoxycarbonyl (R4 -I). These nine compounds are showing good interactions with HTLV-1 PR, with good scoring and well bound inside the binding pocket (Table 3). The better results in the molecular docking inform about the interacting active site residues and the comparison of experimentally available and theoretically predicted is cross validated with docking results in specification of residue capacity to bind ligand atoms. Theoretical prediction of active site may or may not be accurate, so comparison of theoretical prediction with reference to experimentally available and site based molecular interaction results with reporting residues having capacity to interact. In comparison of active sites of experimentally reported theoretically predicted and docking interaction residues results with LEU56, LEU91, LLE100 shows absence of active site property and residues of SER55, GLY58, ASN97 are inspired for active site property showing this residues can also function as active site of HTLV-1 PR..

LSBD Descriptors

Ligand and structure based descriptors are generated to maximize the capabilities of the methods for predicting and rank-ordering the binding affinities of compounds for a given target protein. Here Ligand-receptor complex descriptors are obtained from the structural information and it was calculated by Embrace, Liaison, Prime MM/GBSA, and Qikprop which

are tabulated and used for enhancing the docking results. While based on these results of binding free energy was calculated for each docking pose. From that, compounds which having tendency to execute more -35kcal/mol are considered for next phase of study. The good Gscore, binding energy, and H-bond interaction of this limited screened compounds tabulated in table 3. The screened nine compounds are having enough Gscore, energy parameters and also have tendency to form strong interactions towards the HTLV-1 PR. When comparing these nine compounds with available inhibitors, each compound is having unique pharmacophoric sites, but all compounds are dominated with hydrogen bond acceptor, hydrophilic region and aromatic ring.

For these nine compounds, various energy parameters has been evaluated like electrostatic energy (U_{elec}), van der Waals energy (U_{vdw}), solvation energy (G_{solv}) using embrace and liaison. These are energetics of ligands binding to active site surfaces and able to determine the binding mode of known high-affinity ligands and find new active compounds. The solvent water is used for calculation of binding energy; in specific Ucav is prediction of cavity energy, by using the RADAP (apparent radius of the solvent) and vdw Energy is predicted by using the RAD_HS (hard sphere radius of the solvent). These solvent based energy approaches are physical terms, which are contributed in protein ligand molecular recognition, that computationally predict the energy parameters using Hybrid Monte Carlo simulation with dielectric constant. The energy values are represented in Table 4. shows the energy configuration between the ligand and protein binding; these energies are responsible for binding of new inhibitors with HTLV-1 PR. ADME delivers medicinal chemistry task - PSA, represented in Table 4. This is polar surface area; determine the induction of new lead capacity, into the cell membrane, the new molecules of C and E having PSA more than 140

are eliminated by this medicinal chemistry task.

Molecular recognition

From the nine hit compounds, only seven compounds (A, B, D, F, G, H, I) are filtered through the PSA and these compounds are analyzed for molecular recognition with HTLV-1 PR crystal structure contours and new leads pharmacophore. As these libraries of Purvalanol-A are designed with respect to contours of receptor binding site. These molecular recognition fixes where the compound correctly suitable for molecular environment. To understand the pharmacophoric features and evaluate specific drug–receptor interactions with respect to contours provides strong binding of protein ligand interactions. The interaction identifying important specific drug–receptor interactions between the Purvalanol-A attached with Benzyloxycarbonyl in R4 position having the pharmacophore feature of Acceptor - 6, Donor - 3, Hydrophobic -3, Aromatic -4, which fits best interaction with HTLV-1 PR, in other words, it fits best to contours of HTLV-1 PR. Availability of benzyl group, Hydrophobicity, and stereochemistry of certain functional groups are found to be important for inhibiting to the HTLV-1 PR, The molecular recognition of ligands pharmacophore which is active part of the drug molecule search for contours in receptor active site and binds to it with hydrogen bond interaction. The F**igure 5** clearly mention of HBAR, HBDR, HPR, and HPBR are charged environment created by OPLS-2005 which occupies the benzyloxycarbonyl in R4 position having pharmacophoric feature of A- 6, D 3, H-3, R -4, and so it is shown to have good docking score and binding energy. The R4 – Pivaloyl not filling the molecular recognition due to non-relation of binding contour and ligand pharmacophore sites, (which is represented in **Figure 5**) due to this non relation, the R4 Pivaloyl has lost its capacity to dock with HTLV-1 PR and insufficient binding energy. So the molecular recognition is important in receptor –

ligand interactions, in words of recognition of binding contours of HTLV-1 PR recognize the perfect pharmacophores of new leads gives better docking score, binding energy and compounds which passed through docking and binding energy analyzed with structure and ligand based descriptor analysis for theoretical analysis of activity with respect to contours are having the ability to inhibit the HTLV-1 PR which is target for anti-cancer drug design.

Molecular Dynamics Simulation

The ligand and the receptor complex were further refined by MD simulations for 20 ns. The essential dynamic behavior of HTLV-1 PR protein in a water model was regulated up to 20ns. The Root Mean Square Deviation (RMSD) of HTLV-1 PR backbone structure with respect to the initial conformation was calculated as a function of time period to assess the conformational stability of the protein during the simulations (Figure 6). RMSD of the apo-form was ~0.13 nm after 3.1ns simulation and remained stabilized till the end of simulation. The initial and final confirmation of the protein structure has been morphed with Chimera and analyzed. The Backbone structure of the protein remains same till the end of the simulation and the deviations are very less of 0.3Å. The stable conformation obtained from dynamic studies will enhance the success rate of docking interactions and so we chosen the stable average conformation for the computational part. The RMSD of apo (receptor alone) and the seven holo forms (receptor with ligands) during the MD simulation were shown in figure 6. Similarly, the average RMSD of the Fluroenylmethoxycarbonyl (R1 -A), Phosphate (R2 - B), Benzyloxycarbonyl (R4 - D), Sulfate (R3 -F), Phenyloxycarbonyl (R4- G), Trifluroacetyl (R3 - H) and Fluroenylmethoxycarbonyl (R4 -I) (seven holo-forms) showed ~0.34 nm, ~0.27 nm, ~0.29 nm, ~0.33 nm, ~0.33 nm, ~0.38 nm and ~0.31 nm respectively. The average RMSD of holo forms is higher than the apo form of HTLV-1 PR and both the bound and unbound forms

of simulated structure that remained same till the end of simulation. Ligand bound structures showed deviations, which shows that the new ligands are much active inside the binding pocket. The hydrogen bonds formed are uniformly strong and holds the new lead compounds inside the HTLV-1 PR binding pocket (Figure 7). The seven holo forms (receptor with ligands) during the MD simulation show strong binding and their average hydrogen bond formation is statistically calculated. The average h-bonds of Fluroenylmethoxycarbonyl (R1 -A), Phosphate (R2 - B), Benzyloxycarbonyl (R4 - D), Sulfate (R3 -F), Phenyloxycarbonyl (R4- G), Trifluroacetyl (R3 -H) and Fluroenylmethoxycarbonyl (R4 -I) are 3.8, 3.2, 3.3, 4.8, 2.8, 3.0 and 2.7 respectively. All the new lead compounds are showing average H-bonds of ±3, which is higher than the known inhibitor purvalanol-A. These new compounds on binding with stable potential energy supports that new leads have strong potential to hold inside the binding pocket (Figure 8). Potential energy fluctuations are unseen for all the new compounds. On the whole, the new lead compounds are stable, showing strong interactions, much active and showing binding potential towards HTLV-1 PR. These results suggested that binding of the ligand to the protein showed deviation from their initial position because of adjustments in their configuration but still remains to be bound within the active site of the protein.

Conclusion

Binding of small-molecule ligands to protein active sites is a key objective of drug designing, theoretical interactions associated receptor captures the effects of the ligand interaction from the protein active site with molecular recognition. Inhibition pattern of active

site predicted with HTLV-1 PR, and designed leads compounds of better interaction than Purvalanol-A, which shows up to -10.208523 of docking score, where Purvalanol-A gives -6.702560. The molecular interaction reveals that SER55, GLY58, and ASN97 can also function as active sites, with experimentally reported active sites as analyzes of both theoretical and experimental studies of active site is important for drug designing. Compounds of Purvalanol –A attached R1- Fluroenylmethoxycarbonyl, R2-Phosphito, R3-Sulfato, R3-Trifluroacetyl, R4 – Benzyloxycarbonyl, R4, Phenyloxycarbonyl, R4 – Fluroenylmethoxycarbonyl, shows good molecular level interaction, which results with ranking of top poses through binding energy, docking analysis, structure and ligand based descriptors and molecular dynamics simulations. The compounds which passed in docking interaction as best compound, may fails in binding energy, this is because of not considering the molecular level environment. Finally nine best leads of both docking interaction and binding energy were obtained based on the proper knowledge of both experimental and theoretical active sites, inhibiting HTLV-1 PR, with designed small molecules based on requirement of contours present in active site. The inhibition of protein active site is not like just protein-ligand interaction, the actual phenomenon behind these studies involve with HBAR-A, HBDR-D, HPBR –R, HPR-H, and i.e. protein – ligand interaction deals with back end of contour of active site and ligand pharmacophore interactions. By these results, the interaction between the ligand and protein lies with contours and pharmacophore interactions. The ligand pharmacophore searches appropriate place in binding site and it places well according to respective receptor contour surfaces. This molecular level recognition based on ligand pharmacophore relies on respective contours, and finally this molecular attachment of ligand and receptors fused with hydrogen bond formation.

Declaration of Interest: None

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Legend to Tables

Table 1: *Represents the molecular interaction between the crystal structure of HTLV-PR and known inhibitor Purvalanol-A*

Table 2: *Represents the active site difference in both experimental and theoretical prediction, which is from reported PDB complex 3LIN, 3LIQ, 3LIT, 3LIV, 3LIX, 3LIY crystallographic information of active site is obtained and theoretical prediction is obtained from Sitemap*

Table 3: *Represents the best ligand extracted from molecular docking, binding energy and descriptors, involved and pharmacophore of ligand, which is narrated as A, D, H, N, P, R*

Table 4: *Represents OPLS-2005 based vanderwaals energy, electrostatic energy, solvation energy in ligand binding and cavity energy in presence of hybrid water model as solvent through Hybrid Monte Carlo simulation approach and Polar surface area of selected compounds*

Table 1

Molecular Docking in HTLV-PR (2B7F)		Contour maps Prediction of Purvalanol-A	
Ligand Name	Purvalanol - A	**H-Bond acceptor region(HBAR)**	738.927
Gscore	-6.702560	**H-Bond donor region(HBDR)**	1102.740
No of Interactions	3(A1 and B2)	**Hydrophilic region(HPR)**	1803.663
Interacting Residues	GLY34, ASP 32	**Hydrophobic region(HPBR)**	311.759
Chain A	GLY34	**Metal Binding region(MBR)**	0.000
Chain B	GLY34, ASP 32		

Table 1: *Represents the molecular interaction between the crystal structure of HTLV-PR and known inhibitor Purvalanol-A*

Table 2

Experimental Active sites reported	Theoretical Active site prediction	Docking Interaction Residues	Additional Residues Interaction
ARG10 LEU30	ARG10 LEU30	ARG10 LEU30	SER55
ASP32 GLY34	LEU31 ASP32	ASP32 GLY34	GLY58
ALA35 ASP36	THR33 GLY34	ALA35 ASP36	ASN97
MET37 VAL39	ALA35 ASP36	MET37 VAL39	
LEU56 LEU57	MET37 THR38	SER55 LEU57	
ALA59 LEU91	VAL39 SER 55	GLY58 ALA59	
TRP98 LLE100	LEU56 LEU57	ASN97 TRP98	
	GLY58 ALA59		
	LEU91 ASN97		
	TRP98 LLE100		

Table 2: *Represents the active site difference in both experimental and theoretical prediction, which is from reported PDB complex 3LIN[9], 3LIQ[9], 3LIT[9], 3LIV[9], 3LIX[9], 3LIY[9]***,** *crystallographic information of active site is obtained and theoretical prediction is obtained from Sitemap*

Table 3

Computed values of Protein Ligand interaction								
Ligand Name	**Pharmacophore feature**						**Gscore**	**DG_{bind}**
	A	D	H	N	P	R		
Fluroenylmethoxycarbonyl (R_1 -A)	6	3	4	0	0	5	-8.565992	-42.570530
Phosphate (R_2 - B)	7	5	3	0	0	3	-8.590773	-48.911172
Phosphate (R_4 - C)	8	3	3	1	0	3	-8.520319	-40.469488
Benzyloxycarbonyl (R_4 - D)	6	3	3	0	0	4	-9.017948	-48.872633
Phosphate (R_2 -E)	8	3	3	1	0	3	-8.711677	-44.911172
Sulfate (R_3 -F)	6	2	4	1	0	3	-8.468488	-43.204161
Phenyloxycarbonyl (R_4- G)	6	3	3	0	0	4	-8.812543	-39.448403
Trifluroacetyl (R_3 -H)	5	2	5	0	0	3	-9.141660	-49.290872
Fluroenylmethoxycarbonyl (R_4 -I)	6	3	4	0	0	5	-9.221705	-50.243652

Table 3: *Represents the best ligand extracted from molecular docking, binding energy and descriptors, involved and pharmacophore of ligand, which is narrated as A, D, H, N, P, R*

Table 4

Computed Values of Energies involvement in present of Hybrid water model							
Ligand	**vdW energy**	**Electrostatic energy**	**Solvation energy**	**Liaison (Uvdw)**	**Liaison (Ucav)**	**Liaison (Uele)**	**PSA**
A	-261.210000	-100.380000	185.720000	-81.129000	9.360600	3.667800	97.550000
B	-170.570000	-179.200000	171.360000	-55.465000	5.695100	12.401000	106.606000
C	-201.660000	-61.000000	171.960000	-58.130000	6.230670	18.306000	141.354000
D	-239.860000	-247.760000	300.330000	-66.877000	6.885390	-12.233160	111.677000
E	-166.880000	-142.200000	153.160000	-55.066000	5.257900	-0.862400	140.425000
F	-210.970000	-88.990000	159.590000	-62.493000	7.755942	0.727800	119.188000
G	-225.880000	-114.140000	217.530000	-68.132000	5.880370	13.594400	103.596000
H	-216.380000	-33.040000	110.010000	-57.772000	4.046390	-0.383400	76.459000
I	-256.240000	-176.750000	262.440000	-71.867000	7.368280	-4.637400	102.291000

Table 4: *Represents OPLS-2005 based vdW energy, electrostatic energy, solvation energy in ligand binding and cavity energy in presence of hybrid water model as solvent through Hybrid Monte Carlo simulation approach and Polar surface area of selected compounds*

Legend to Figures

Figure 1: *Represents the contour based ligand designing, from the Structure of HTLV-1 protease, colored in chain difference -monomer subunit is shown in a red and grey ribbon and contours are analyzed for ligand rearrangement with pharmacophore rearrangement.*

Figure 2: *The Reported compounds Purvalanol-A showing interactions with both chain A (GLY34) and chain B (ASP32, GLY34) amino acids*

Figure 3: *Visualization of contour maps showing the active site environment of HTLV PR protein, which includes Hydrogen Bond Acceptor Region (HBAR-red mesh), Hydrogen Bond Donor Region (HBDR-blue mesh), Hydrophilic Region (HPR-green mesh) and Hydrophobic Region (HPBR-yellow mesh) in the presence of Purvalanol-A.*

Figure 4: *Molecular docking interactions of nine best lead compounds passed in both docking score and binding energy calculations (A = R_1 Fluroenylmethoxycarbonyl, B = R2 Phosphito, C = R4 Phosphato, D = R4 Benzyloxycarbonyl, E = R2 Phosphato, F = R3 Sulfato, G = R4 Phenyloxycarbonyl, H = R3 Trifluroacetyl, I = R_4 Fluroenylmethoxycarbonyl. These nine compounds are having good Gscore, binding energy, activity, Hbond interaction.)*

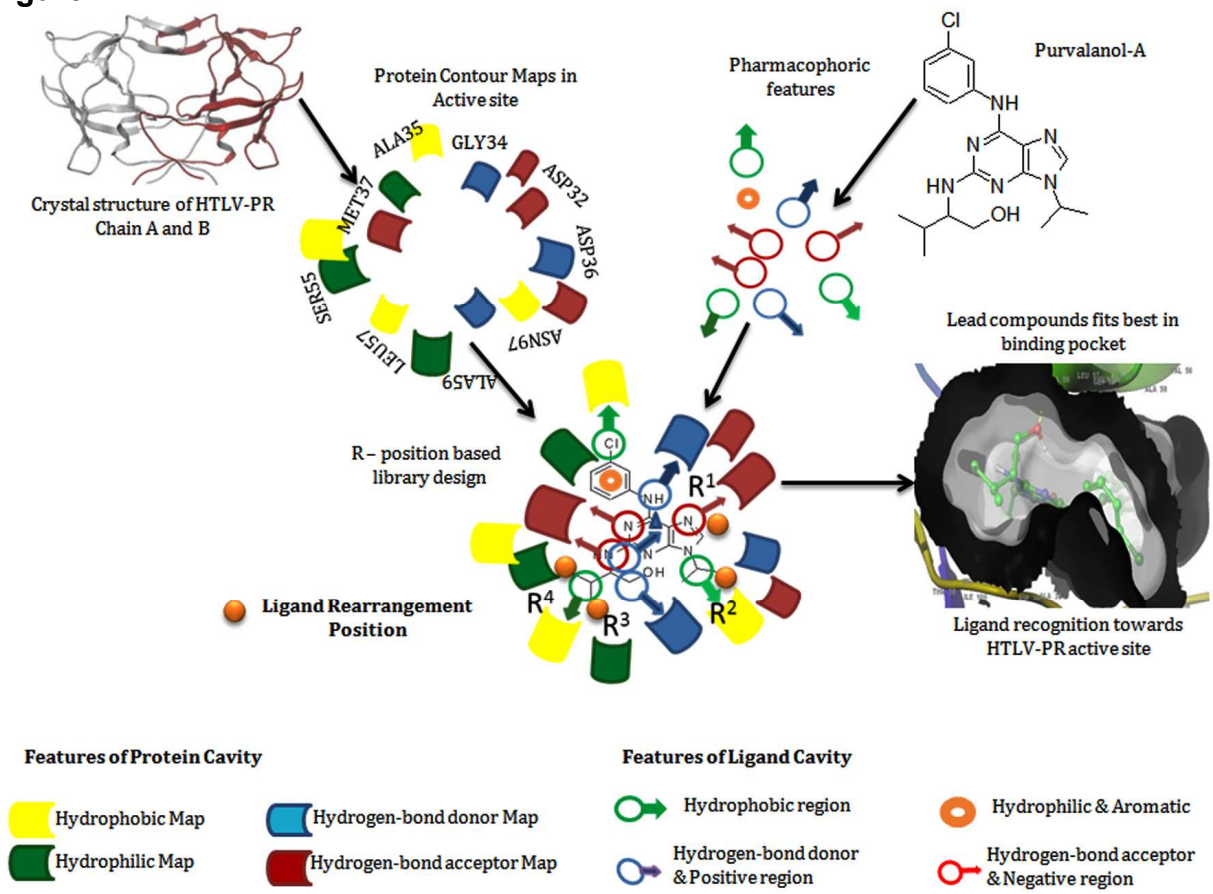
Figure 5: *Molecular recognization of Protein contours interactions with respective ligand pharmacophoric sites, ensures strong interactions and binding potential towards new leads of HTLV-1 PR inhibitors. (a) Surface contours present in HTLV-1 PR active site. (b)Pharmacophoric sites present in the purvalanol A. (c) Red color mesh protein contours interactions with hydrogen bond acceptors of ligands. (d) Blue color mesh protein contours interactions with hydrogen bond donors of ligands. (e) Hydrophilic Region - green mesh interactions with hydrophilic and aromatic rings of ligands. (f) Hydrophobic Region of yellow mesh in proteins interacts with hydrophobic regions of ligand pharmacophoric sites.*

Figure 6: *RMSD graph for HTLV PR apo and ligand complex for the timescale event of 20ns showing with average mean variations respectively*

Figure 7: *Hydrogen bond interactions of Ligand bound complex structures in the timescale of 20ns and with average h-bond interactions respectively*

Figure 8: *Potential energy of ligands exhibited during the molecular dynamics simulation for the timescale event of 20ns*

Figure 1



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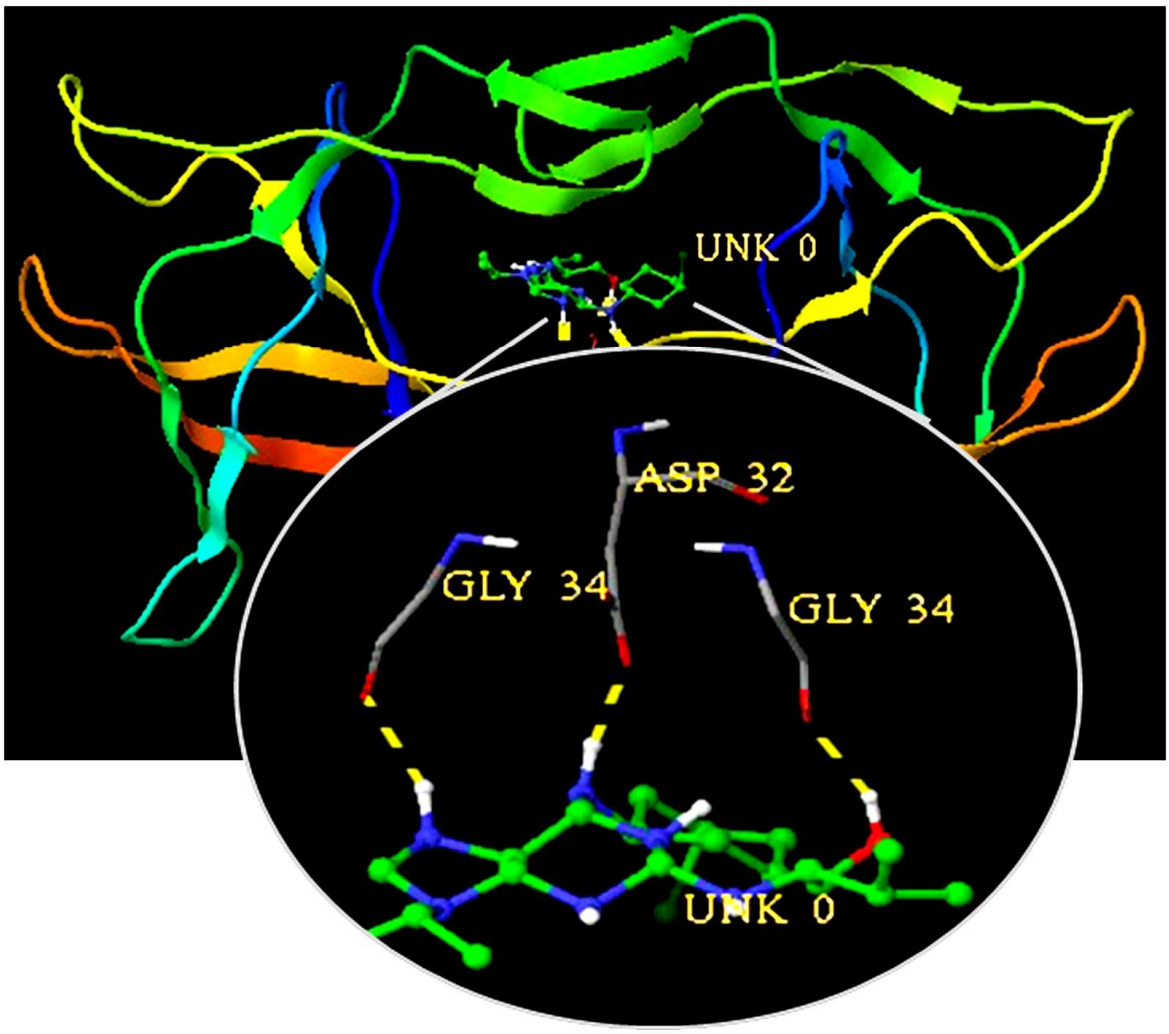

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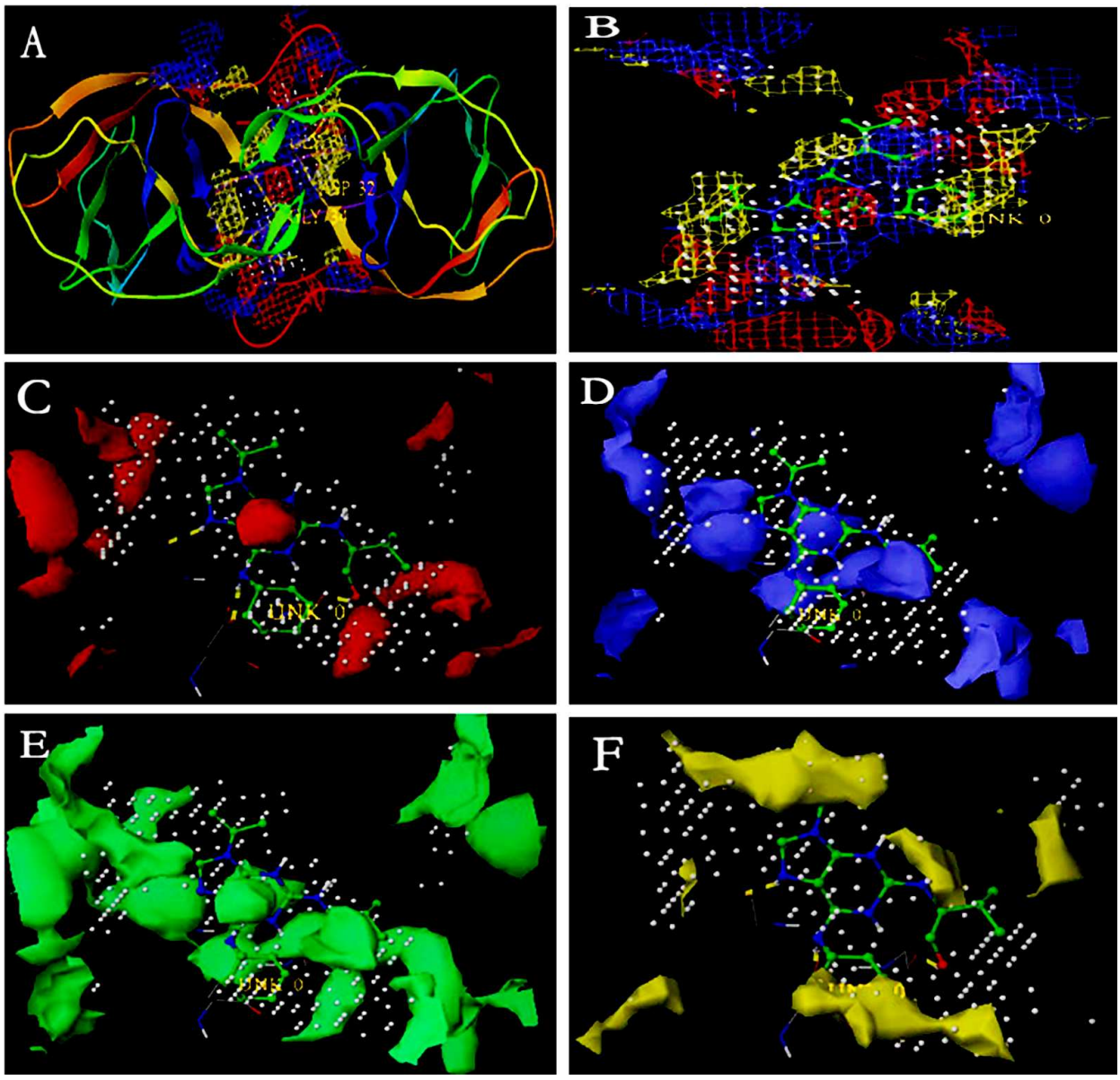

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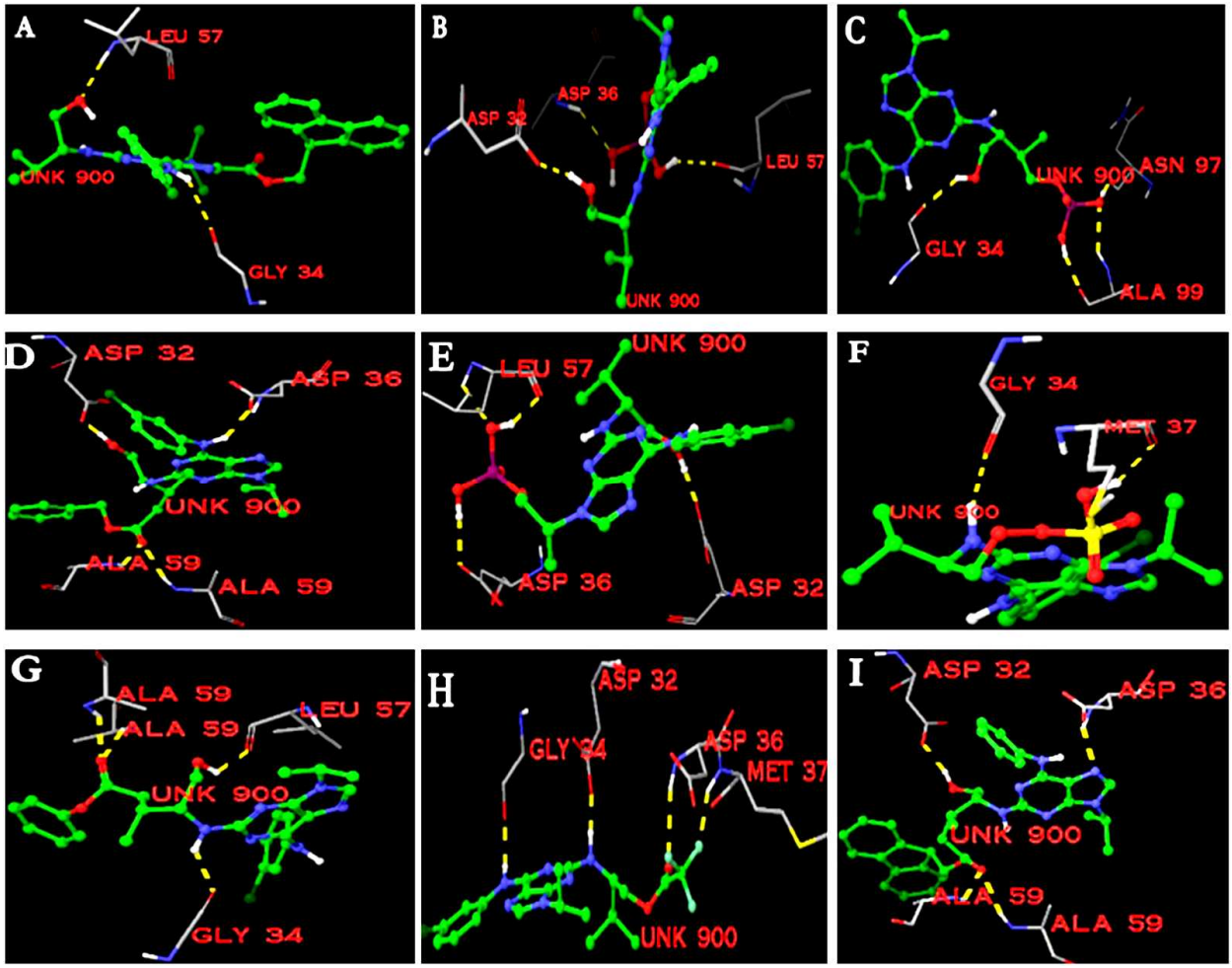
Figure 4



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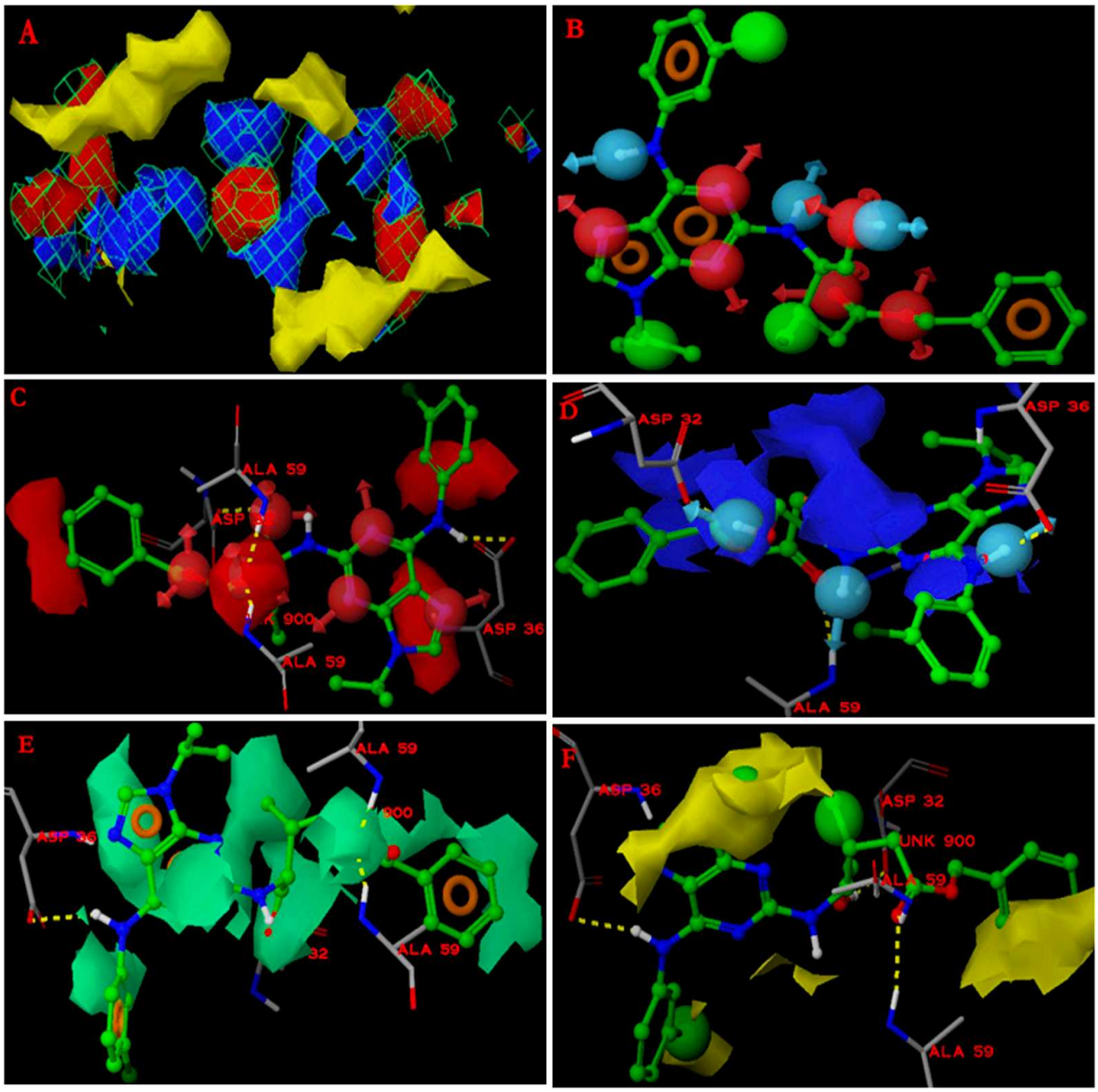

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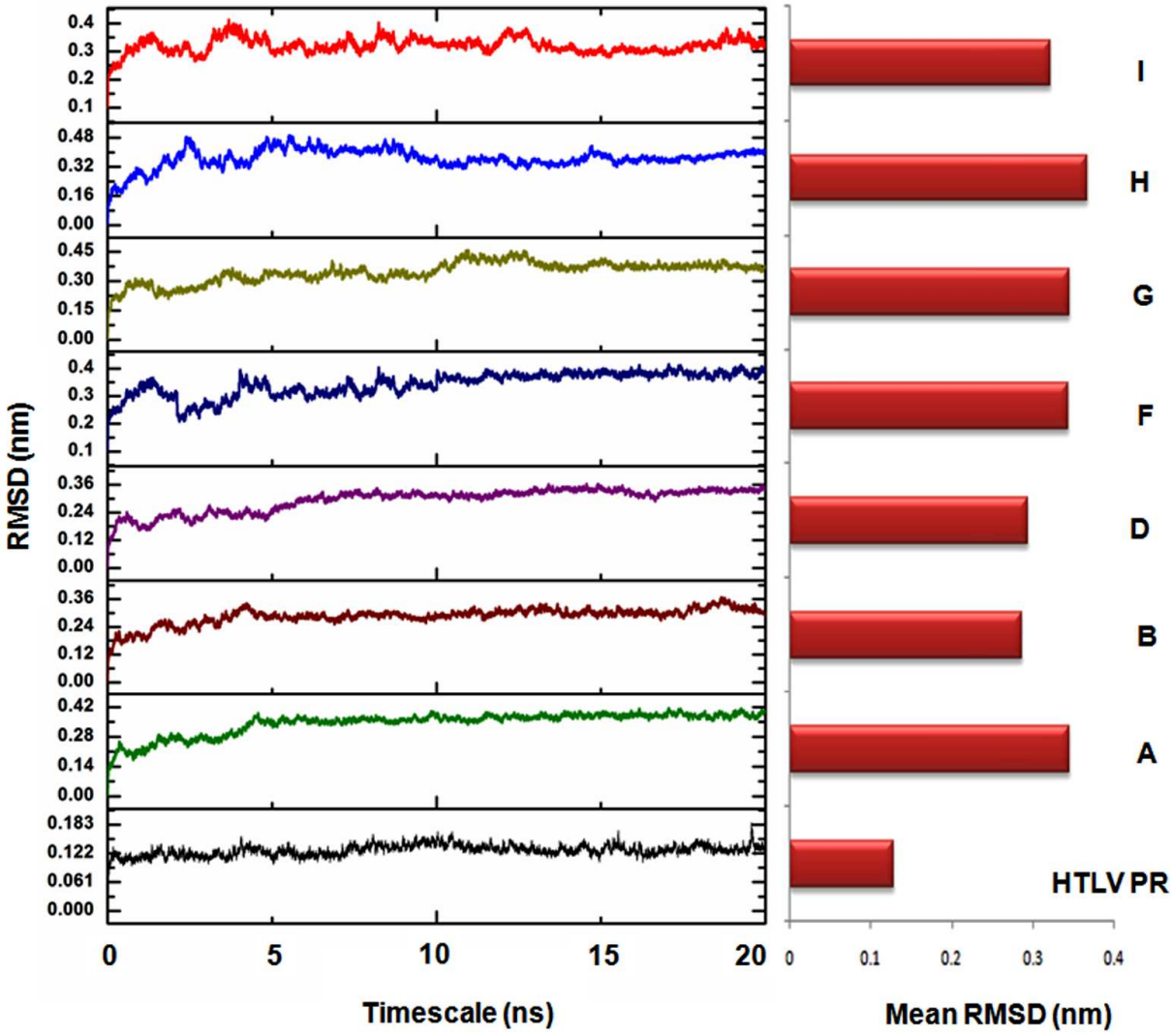


Figure 6: RMSD graph for HTLV PR apo and ligand complex for the timescale event of 20ns showing with average mean variations respectively

Figure7

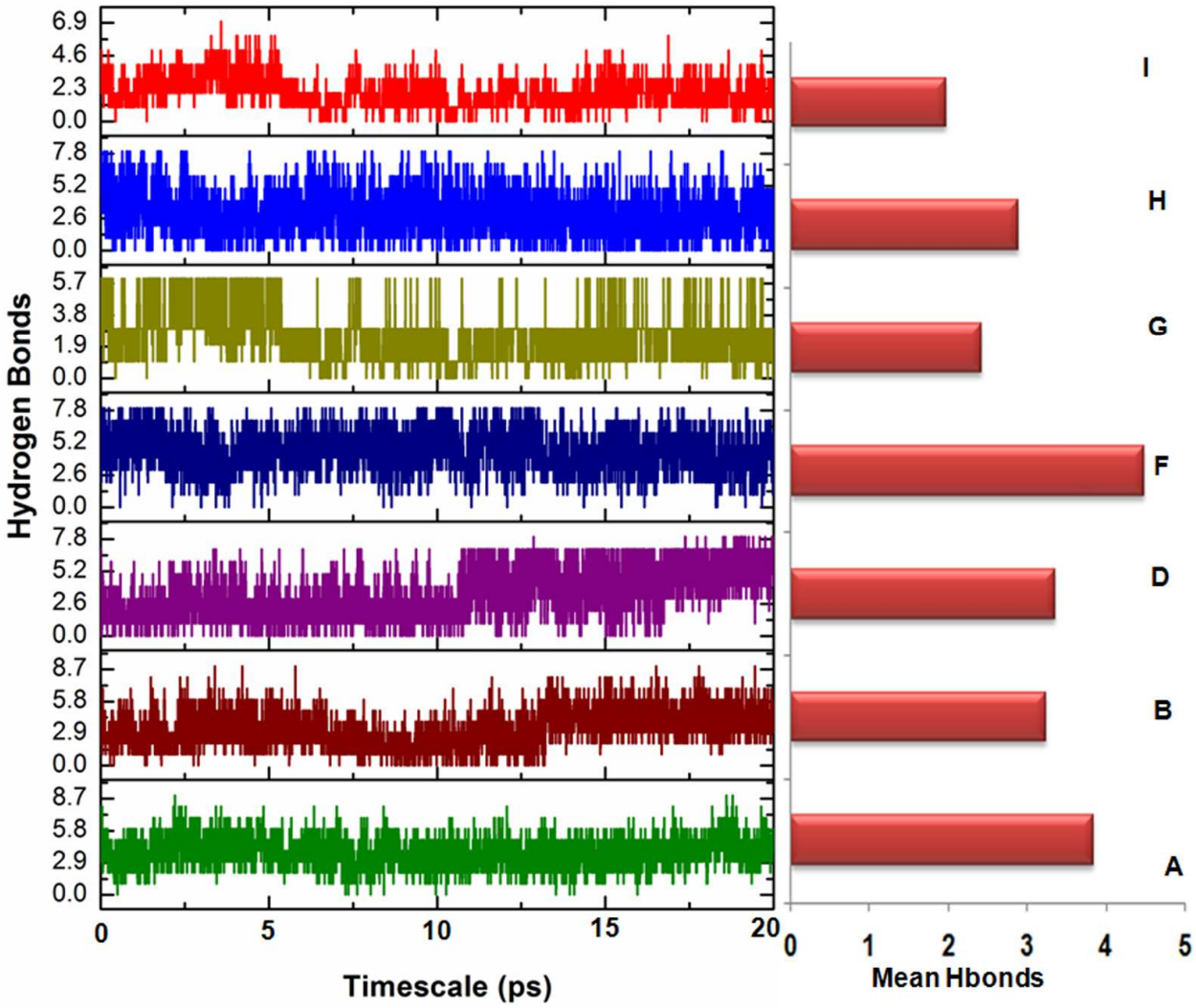
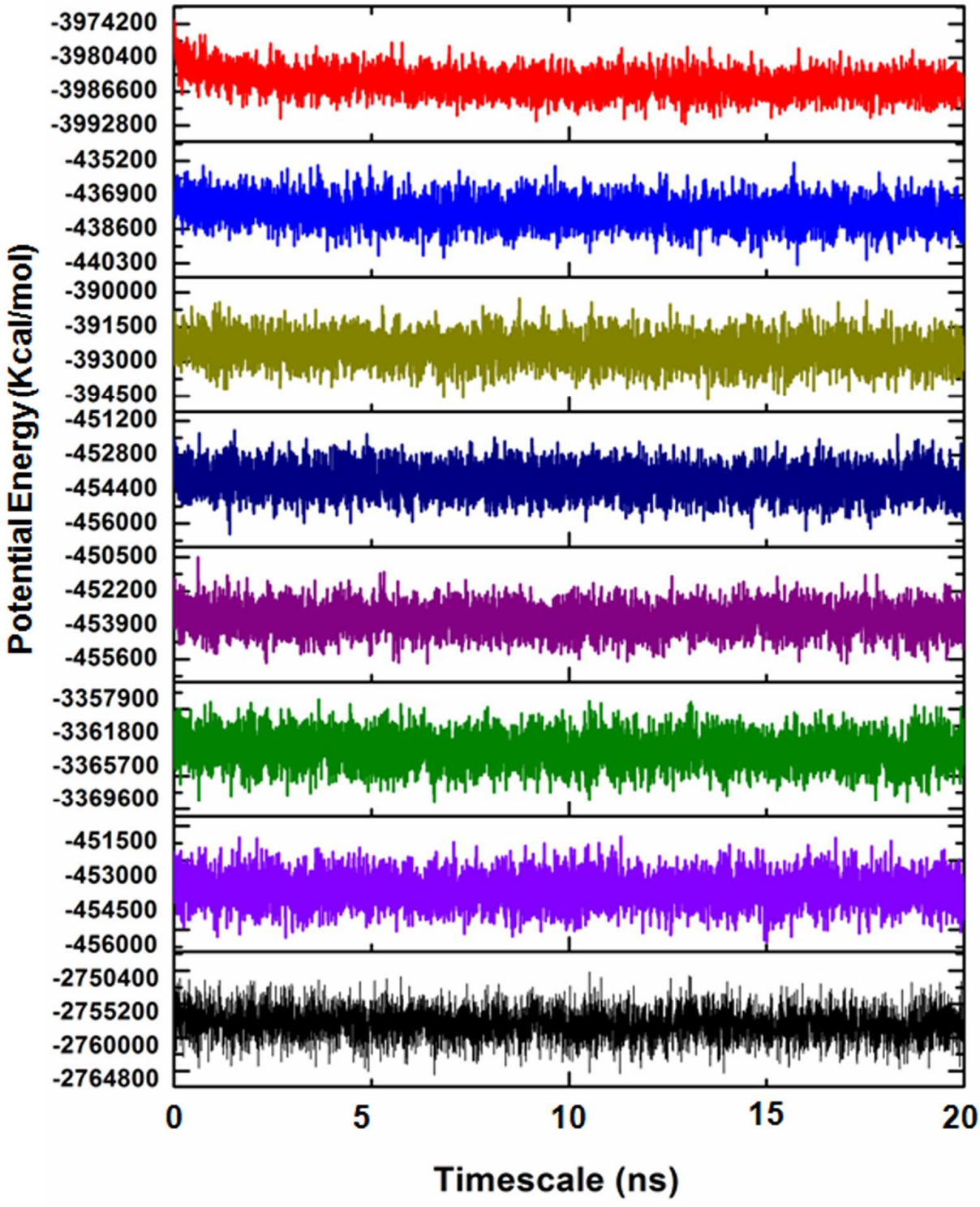


Figure 7: Hydrogen bond interactions of Ligand bound complex structures in the timescale of 20ns and with average h-bond interactions respectively

Figure 8



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