

RESEARCH ARTICLE

Structure-based pharmacophore modeling, high-throughput screening, and molecular dynamics identify a novel DrugBank-derived HIV-1 protease inhibitor

Ali Sultan Khazaal Nazal¹, Amjed Majid Rashid Alrashedi^{1*},
Lamia Abdultef Risan Al-Iessa², Mustafa Abdulkadhim Hussein Ali Rabeea²

1 Department of Biochemistry, Faculty of Science, Kashan State University, Kashan, Iran, **2** Department of Chemistry, Faculty of Sciences, Kufa University, Najaf Al-Ashraf, Iraq

* amjad93majid11@gmail.com



OPEN ACCESS

Citation: Khazaal Nazal AS, Rashid Alrashedi AM, Risan Al-Iessa LA, Ali Rabeea MAH (2026) Structure-based pharmacophore modeling, high-throughput screening, and molecular dynamics identify a novel DrugBank-derived HIV-1 protease inhibitor. PLoS One 21(6): e0336765. <https://doi.org/10.1371/journal.pone.0336765>

Editor: Soumendranath Bhakat, AlloTec Bio, UNITED STATES OF AMERICA

Received: October 30, 2025

Accepted: May 27, 2026

Published: June 12, 2026

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0336765>

Copyright: © 2026 Khazaal Nazal et al. This is an open access article distributed under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/)

Abstract

HIV-1 protease (PR) is required for viral maturation and is a major antiretroviral target. In this study, a structure-based pharmacophore modeling, drug repurposing, docking, and molecular dynamics (MD) was applied to discover new PR inhibitors. A pharmacophore model was generated from the HIV-1 PR–3TL complex (PDB 3KFP) and used to screen the DrugBank library and hit identification. The identified hit and reference 3TL were docked into PR, and each complex was simulated for 100 ns. Key metrics, including binding energy, RMSD, RMSF, SASA, hydrogen bonds, salt bridges, PCA were analyzed and compared. Through the screening, one hit, HONH-BENZYLALONYL-L-ALANYLGLYCINE-P-NITROANILIDE (DrugBank Accession Number: DB07434) (BAN), was identified. Docking predicted BAN's binding energy (−7.51 kcal/mol) slightly better than 3TL (−7.05 kcal/mol). MD showed BAN established a dense H-bond network, including Asp25, and a highly favorable total interaction energy (−69 kJ/mol). However, BAN binding significantly increased protease flexibility. BAN-bound PR had higher backbone RMSD (0.34 nm) and RMSF (0.215 nm) than 3TL-bound (0.22 nm, 0.121 nm), and disrupted salt bridges that remained stable with 3TL. PCA revealed BAN-bound PR sampled a larger conformational space. SASA changes were minor in all systems. BAN binds HIV-1 PR with affinity comparable to 3TL but via a distinct mechanism, stronger polar interactions accompanied by greater protein flexibility. These results, supported by recent literature, suggest BAN as a novel scaffold for PR inhibition. Experimental validation of BAN's inhibitory activity is warranted.

[License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data availability statement: All relevant data are within the paper and its [Supporting information](#) files.

Funding: The author(s) received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Human immunodeficiency virus type 1 (HIV-1) protease (PR) is a homodimeric aspartyl protease whose catalytic dyad (Asp25/Asp25') and mobile flaps mediate cleavage of viral polyproteins into mature proteins [1,2]. Blocking this cleavage with protease inhibitors (PIs) halts virion maturation and dramatically improved AIDS outcomes [1]. Indeed, ten PIs like darunavir and lopinavir are FDA-approved for HIV therapy [2,3]. However, rapid viral mutation leads to multi-drug resistant (MDR) HIV-1 strains, limiting long-term PI efficacy [4–7]. Thus there is an urgent need for novel HIV-1 PR inhibitors, ideally identified by rapid, cost-effective methods.

Structure-based drug design (SBDD) has a proven history in HIV-1 PR inhibitor development [8]. Early peptidomimetic PIs were derived from crystal structures of PR with substrates and inhibitors, demonstrating the power of structural data in lead optimization [1,8]. Modern computational techniques, including pharmacophore modeling [9,10], structure-, ligand-, and fragment-based drug discovery approaches [11–14], and molecular dynamics (MD) [11,15] have increasingly enabled the exploration of vast compound libraries and in silico evaluation of binding at atomic detail [16,17]. High-throughput docking of compound libraries, including FDA-approved drugs or fragment libraries against HIV-1 PR has successfully identified new scaffolds. In this regard, a recent study screened thousands of PubChem compounds via pharmacophore filtering and rigid-receptor docking to uncover novel hits with comparable or better predicted affinity than approved PIs [16,18].

In these computational pipelines, protein–ligand docking provides an initial binding score and pose prediction, while MD simulations validate and refine these complexes by sampling conformational flexibility and estimating binding energetics over time [16,18]. Key metrics from MD simulation, such as root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), solvent-accessible surface area (SASA), hydrogen-bond occupancy, salt-bridge persistence, and principal component (PCA) analyzes, quantify binding stability, protein mobility, and dominant motions. For HIV-1 PR, MD studies have revealed that stable inhibitors typically lock the flaps in closed conformations and maintain strong polar contacts with catalytic residues [16,18]. In contrast, weak or atypical binders can allow greater flap mobility and reorganize intramolecular interactions. Recent efforts have even applied artificial intelligence (AI) and machine learning to prioritize candidate molecules before docking, underscoring the synergy of modern computational methods [17,19].

Here we combined structure-based pharmacophore modeling, DrugBank virtual screening, molecular docking, and extended MD simulations to discover and evaluate a DrugBank-derived HIV-1 PR ligand relative to a known reference inhibitor, 3TL. The goal was to interpret the binding energetics and dynamics in the context of current literature. We analyze RMSD/RMSF convergence, SASA, ligand–protein interaction energy, hydrogen bonding patterns, intramolecular salt bridges, and principal components to assess binding stability and conformational effects. By comparing our findings to recent studies, we aimed to explain how the novel hit engages HIV-1 PR and how it differs from the reference inhibitor, thereby guiding future optimization.

Materials and methods

Data gathering of preprocessing

HIV-1 Protease (PR), complexed with a reference inhibitor, 3TL or as we call it from now on ‘3TL’ was obtained from the Protein Data Bank (PDB; <https://www.rcsb.org/>) using the PDB ID 3KFP. This structure was derived from a fragment-based crystallographic screen of wild-type NL4-3 HIV Protease [20]. The crystal structure was prepared for computational analysis as described before [21–26]. Accordingly, all non-essential molecules, including water molecules, co-solvents, and ions, were removed from the PDB file. The coordinates of the PR monomer and the co-crystallized inhibitor/fragment were retained. Hydrogen atoms and appropriate bond orders were added to the polar residues of protein structure and the bound ligand using UCSF Chimera and Avogadro, respectively. The prepared protein structure was used to define the binding site for subsequent pharmacophore modeling, molecular docking and dynamic simulation.

Structure-based pharmacophore model generation

A structure-based pharmacophore model was generated by analyzing the specific non-covalent interactions between the key reference inhibitor 3TL and the HIV-1 Protease residues. Accordingly, LigandScout software v4.4.7 software was used for preparing the pharmacophore as described before [9]. Briefly, the 3D coordinates of the prepared HIV-1 PR–inhibitor complex (PDB ID 3KFP) were imported into LigandScout. The software was employed to automatically identify and annotate essential pharmacophoric features, which include Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), Hydrophobic centers, and Aromatic Ring features. This was based on the physicochemical properties and spatial arrangement of the co-crystallized ligand and the surrounding amino acid residues in the binding pocket. The generated pharmacophore model was manually inspected and refined to ensure chemical relevance and maximize selectivity. Accordingly, features contributing to critical anchor points, such as those forming hydrogen bonds with the backbone or conserved catalytic residues like Asp25/Asp25’ in HIV PR), were retained.

Preparation of chemical library and screening

The selected pharmacophore model was utilized to perform a high-throughput virtual screening. In this regard, a comprehensive compound library of DrugBank database was prepared for screening based on the previously reported study [10]. Compounds were subjected to conformational sampling and energy minimization using LigandScout’s integrated features with the Omega best algorithm to generate a set of energetically favorable 3D conformers for each molecule [9].

The generated pharmacophore model was used as a spatial filter to screen the prepared DrugBank library. The process identified compounds whose conformers could be successfully mapped onto all of the pharmacophoric features in the model. The screened compounds were ranked based on their Pharmacophore-Fit Score, which quantifies how well the compound’s features map spatially onto the pharmacophore query. The top-scoring compounds were designated as “hit” compound(s) for further analysis, such as molecular docking and MD simulations.

Molecular docking

Molecular docking studies were conducted to validate the predicted binding affinity and conformation of the reference and pharmacophore-matched hit compounds within the HIV-1 PR active site. Docking was performed using AutoDock Vina v1.2.3 [27,28] via the UseGalaxy server (accessible at <https://usegalaxy.eu>) [29,30]. The receptor (HIV-1 PR) was treated as rigid, and the hit ligand(s), in their energy-minimized 3D conformations, were treated as flexible. A grid box parameter was defined using MGLTools v1.5.7 to encompass the entire pharmacophore features visually. The Vina scoring function was used to generate nine distinct docking poses for each reference 3TL and hit(s) compounds. The final lead compounds were prioritized for MD simulation.

Molecular dynamics simulations

MD simulations of HIV protease apo and holo systems, including protease complexed with 3TL and the hit compound were carried out using the GROMACS v2022 software package with the AMBER99SB force field [31]. The Ligands' topology was generated using the AnteChamber Python Parser (acpype) package Version 2021.02.05.22.15 [32]. The parameterization utilized the General Amber Force Field (GAFF) v21.10 for describing intra-molecular interactions. Atomic partial charges were derived using the AM1-BCC (Austin Model 1-Bond Charge Correction) method. This two-step approach first calculates the electrostatic potential-derived charges using the semi-empirical AM1 Hamiltonian, followed by empirical bond charge corrections to ensure charges accurately reflect molecular polarity [33–35]. The initial protein structure was embedded in a cubic periodic box with a minimum distance of 1.0 nm between the solute and the box boundary. The system was solvated with 10,139 water molecules using the TIP3P water model. To ensure charge neutrality, an appropriate number of counter-ions (Na^+/Cl^-) were added.

Prior to dynamics, energy minimization was performed using the steepest descent algorithm until the system reached a maximum force below $1000 \text{ kJ}\cdot\text{mol}^{-1}$, removing steric clashes and unfavorable contacts. Following energy minimization, the systems were equilibrated in two successive phases. First, a constant-volume (NVT) ensemble was applied for 50 ps using a leap-frog integrator with a 2 fs timestep, during which the system temperature was maintained at 300 K with a V-rescale thermostat applied separately to protein and solvent groups ($\tau = 0.1 \text{ ps}$). All bonds were constrained using the LINCS algorithm, and electrostatic interactions were treated with the Fast smooth Particle Mesh Ewald (PME) method with a 1.0 nm real-space cutoff, while van der Waals interactions were truncated at 1.0 nm using the Verlet scheme. This was followed by 50 ps of constant-pressure (NPT) equilibration, employing the Parrinello–Rahman barostat with isotropic coupling at 1 bar ($\tau_p = 2.0 \text{ ps}$, compressibility = $4.5 \times 10^{-5} \text{ bar}^{-1}$). During both phases, periodic boundary conditions were applied in all three dimensions, and long-range dispersion corrections were included for energy and pressure. Following equilibration, a 100 ns production MD simulation was performed under periodic boundary conditions in all three dimensions. Electrostatics were computed using the Fast smooth Particle Mesh Ewald (PME) electrostatics method with a real-space cutoff of 1.0 nm, while van der Waals interactions were treated using the Verlet cutoff scheme, with long-range dispersion corrections applied to both energy and pressure. Coordinates were saved every 10 ps for subsequent analyses.

Trajectory analysis

To statistically evaluate the conformational stability and dynamics of the simulated systems [21], key metrics were calculated from the convergence production phase of the trajectories. For all time-series data the analysis included the Mean and Standard Deviation (StDev) to assess central tendency and spread, the Minimum (Min) and Maximum (Max) values to describe the range of motion, and the Standard Error of the Mean (SEM) to quantify the precision of the mean estimate. Furthermore, to focus specifically on the stable molecular dynamics, the Production Mean (ProdMean) and Production Standard Deviation (ProdStDev) were calculated using the last 80% of the simulation time. For the residue-specific data (RMSF), which is not a time-series, the overall Mean, StDev, SEM, Min, and Max were calculated across all residues to quantify the average and range of local flexibility. All statistical results are presented in a structured text file for direct comparison (see supplemental material [S1 Table](#)).

Convergence evaluation. To assess the stability and convergence of each molecular dynamics simulation, the production phase of each trajectory was analyzed. Following standard practice in biomolecular simulations, the initial 20% of each trajectory was designated as the equilibration period and excluded from statistical analysis. The remaining 80% (production phase) was used for all quantitative comparisons. For time-dependent metrics, including RMSD, SASA, and interaction energy, the mean, standard deviation, and standard error of the mean (SEM) was calculated over the production phase.

Hydrogen bond analysis. Hydrogen bond (H-bond) analysis was performed using the Hydrogen Bonds plugin of Visual Molecular Dynamics (VMD). To ensure accurate detection of hydrogen bonds throughout the simulation, the following parameters, including all frames of the 100 ns trajectory, donor-acceptor distance cutoff equal to 3.0 Å, angle cutoff of 20°, selection type of both donors and acceptors, enabled update selections every frame, and disabled polar atoms restriction (all atoms considered) were considered.

Principal component analysis. PCA was performed to characterize the essential motions of the system throughout the molecular dynamics (MD) simulation. The covariance matrix of atomic positional fluctuations was calculated after least-squares fitting of the trajectory to the reference structure to remove overall translational and rotational motions. Eigenvalues and eigenvectors were obtained by diagonalizing the covariance matrix using GROMACS tools, and the eigenvalues were used to estimate the contribution (variance explained) of each principal component (PC) to the total motion. The first four PCs, were used for further analysis. The percentage of variance explained by each PC was calculated as [36]:

$$\text{Variance} (PC_i) = \frac{\lambda_i}{\sum_{j=1}^N \lambda_j} \times 100$$

where λ_i is the eigenvalue of the i^{th} PC and N is the total number of components.

Salt bridge analysis. Salt bridge interactions were analyzed to evaluate the stability and persistence of electrostatic contacts during the MD simulation. The analysis was performed using the Salt Bridges Plugin (version 1.0) implemented in VMD over the entire trajectory. Protein residues were selected as the target atom group, and salt bridges were identified based on an oxygen–nitrogen distance cutoff of 3.2 Å, with selections updated for each frame. The descriptive statistics, including the mean, minimum, maximum, and standard deviation of distances, as well as the occupancy percentage, defined as the fraction of trajectory frames in which the oxygen–nitrogen distance was ≤ 3.2 Å.

Statistical analysis

Statistical comparisons between systems were performed using hypothesis tests appropriate for the data distribution and sample size. For pairwise comparisons of production-phase means, Welch's t-test was applied. When normality could not be assumed, the non-parametric Mann–Whitney U test was used. For residue-wise RMSF comparisons involving more than two groups, the Kruskal–Wallis test was employed. All tests were two-tailed, and a significance threshold of $p < 0.05$ was adopted. In addition to significance testing, descriptive statistics, including median, interquartile range (IQR), minimum, maximum, and quartiles, were computed for each system and metric to provide a comprehensive overview of the data distribution. The analyzes were performed by MS-Office Excel v2019. All analyzes are presented in [S1 Table](#).

Inclusivity in global research

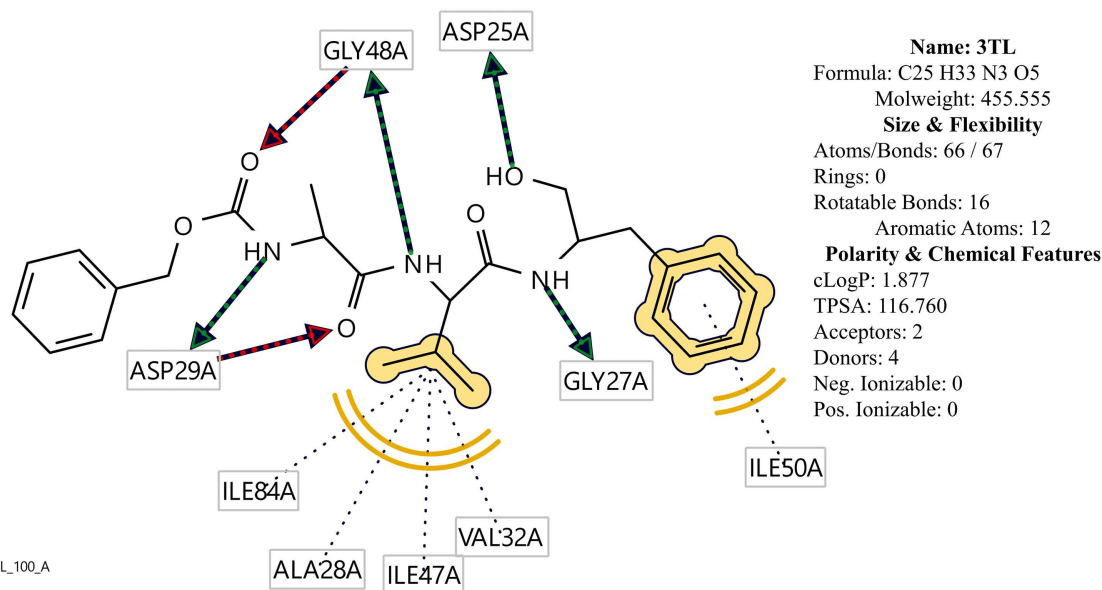
Additional information regarding the ethical, cultural, and scientific considerations specific to inclusivity in global research is included in the Supporting Information ([S2 Checklist](#)).

Results

Pharmacophore-based screening

The pharmacophore extraction of 3TL in its complex with the HIV-1 protease resulted in eight pharmacophore features including, two hydrophobic regions, two H-bond acceptors, four H-bond donors sites. Virtual screening of the Drugbank library with 9.2K molecules has resulted in identification of one hit compound with the generic name of HONH-BENZYLALONYL-L-ALANYLGLYCINE-P-NITROANILIDE (DrugBank Accession Number: DB07434). The relative pharmacophore-fit score was 0.93, matching all 3TL-pharmacophore extracted features ([Fig 1](#)). The

3TL_100_A



UNL_100_A

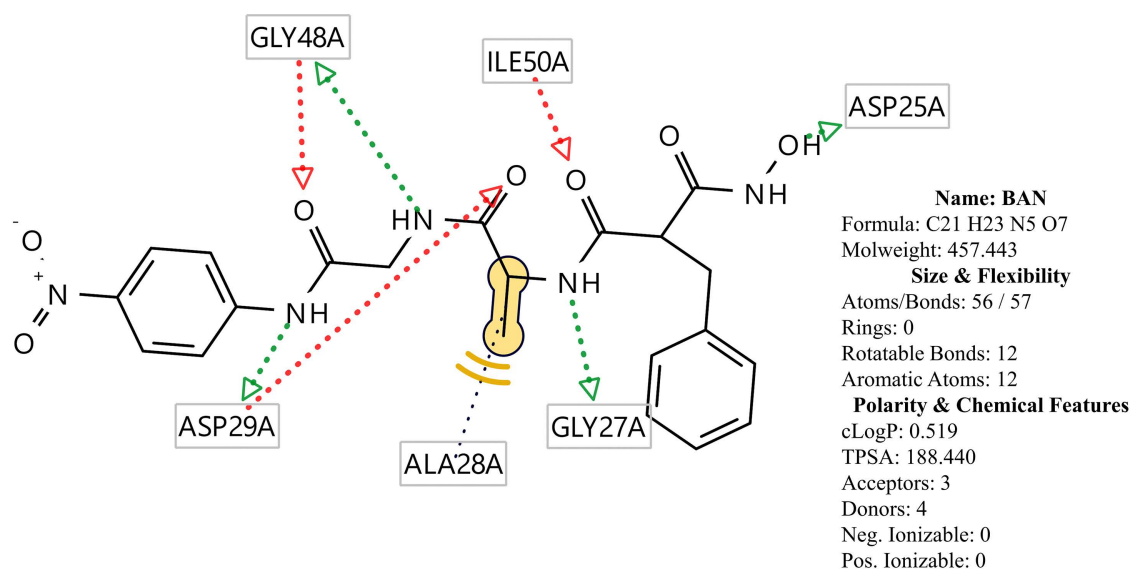


Fig 1. Matching of two reference 3TL compound and the hit Ban compound within the pharmacophore in the HIV-1 protease. Near amino acid residues are depicted. Yellow spears are hydrophobic regions, Green and Red arrows are H bond donors and acceptors, respectively.

<https://doi.org/10.1371/journal.pone.0336765.g001>

compound, which from now on we call it 'BAN', was composed of 200 conformers that its 40th was match with all pharmacophore features with the energy of 98.344.

Computational affinity measurements

Molecular docking of the identified hit compound, BAN, and the reference HIV-1 protease inhibitor 3TL was performed using AutoDock Vina to evaluate their binding affinities and interaction profiles within the protease active site. The docking

results are summarized in terms of binding energy (Vina score), intermolecular and intramolecular contributions, unbound energy, and torsional flexibility. The binding affinity predicted for BAN was $-7.51 \text{ kcal}\cdot\text{mol}^{-1}$, which is slightly more favorable than the reference inhibitor 3TL ($-7.05 \text{ kcal}\cdot\text{mol}^{-1}$). This indicates that BAN is capable of achieving a binding energy comparable to the established inhibitor, suggesting potential for inhibitory activity against HIV-1 protease. The difference, while modest ($\sim 0.46 \text{ kcal}\cdot\text{mol}^{-1}$), may reflect variations in the electrostatic stabilization and conformational flexibility of the two ligands.

The decomposition of binding energies revealed that BAN exhibited an intermolecular interaction energy (INTER) of $-9.75 \text{ kcal}\cdot\text{mol}^{-1}$, comparable to 3TL ($-9.69 \text{ kcal}\cdot\text{mol}^{-1}$). This suggests that both ligands establish similar levels of stabilizing contacts with the protease binding pocket. However, BAN demonstrated a slightly reduced intramolecular energy (INTRA: $-3.41 \text{ kcal}\cdot\text{mol}^{-1}$) compared to 3TL ($-3.84 \text{ kcal}\cdot\text{mol}^{-1}$), implying that BAN adopts its bound conformation with less energetic penalty. This may contribute to its marginally stronger overall binding score. Unbound energy values were also lower for BAN ($-1.04 \text{ kcal}\cdot\text{mol}^{-1}$) compared with 3TL ($-1.35 \text{ kcal}\cdot\text{mol}^{-1}$), reinforcing the observation that BAN achieves stable binding with minimal conformational destabilization relative to its free state.

Analysis of torsional flexibility showed that BAN possessed 12 active torsions, whereas 3TL exhibited 16 active torsions. The reduced number of rotatable bonds in BAN indicates a lower degree of conformational entropy loss upon binding, which may favor its docking score despite its smaller molecular complexity compared to 3TL. Importantly, this suggests that BAN is able to maintain binding efficacy while imposing a lower entropic cost on the system. These findings highlight the need for more investigation into highlighting BAN as a potent inhibitor of HIV-1 protease through MD simulation as of follow.

MD simulation

The stability and dynamics of the target protein, both in its apo form of protease and in complex with ligands 3TL and BAN, holo systems, were evaluated over a 100 ns MD simulation. Analyses were focused on HIV-1 protease stability with RMSD, local residue flexibility with RMSF, protein SASA, specific ligand-protein affinity or interaction energy, hydrogen binding, salt-bridge, and PCA.

Conformational stability and overall trajectory convergence. The RMSD of the protein backbone was monitored to assess the structural stability and convergence of the three simulated systems (Fig 2). The Holo 3TL system exhibited the highest stability and fastest convergence. After an initial slight adjustment in the first 20 ps, the system rapidly stabilized, maintaining a low average backbone RMSD of $\approx 0.223 \text{ nm}$ during the production phase ($>20 \text{ ns}$). The low standard deviation of $\sigma = 0.026 \text{ nm}$ (Table 1) indicates minimal structural fluctuation relative to the starting conformation.

The Holo BAN system demonstrated higher fluctuation and less stability compared to the 3TL complex, oscillating between 0.25 nm and 0.45 nm throughout the production phase. The mean RMSD was substantially higher at 0.342 nm ($\sigma = 0.037 \text{ nm}$). This larger deviation suggests that the binding of the BAN ligand induces a greater conformational change or results in a less rigid final complex structure. The Apo-protease system displayed stability characteristics intermediate between the two holo systems, converging around a mean RMSD of 0.318 nm ($\sigma = 0.041 \text{ nm}$). Although the Apo system's mean is lower than Holo BAN, its larger standard deviation indicates slightly broader conformational sampling. Accordingly, the Holo 3TL complex achieves the most stable conformation, suggesting the 3TL ligand is highly effective at rigidifying the protein structure compared to both the Apo form and the Holo BAN complex.

Local flexibility analysis. RMSF was calculated per residue to identify regions of local flexibility and how ligand binding affects these dynamics (Fig 3). The RMSF for the Apo-protease and Holo 3TL complex were remarkably similar, with a low overall mean RMSF of 0.137 nm and 0.121 nm , respectively (Table 2). This indicates that the 3TL ligand binds without drastically altering the intrinsic flexibility profile of the Apo protein. In contrast, the Holo BAN system exhibits significantly ($p\text{-value} < 0.05$) higher RMSF values across multiple loop regions, particularly around the active site or domain interfaces (residue indices $\approx 50 - 250$ and $\approx 750 - 950$, and a large peak near residue 1500). The mean RMSF for

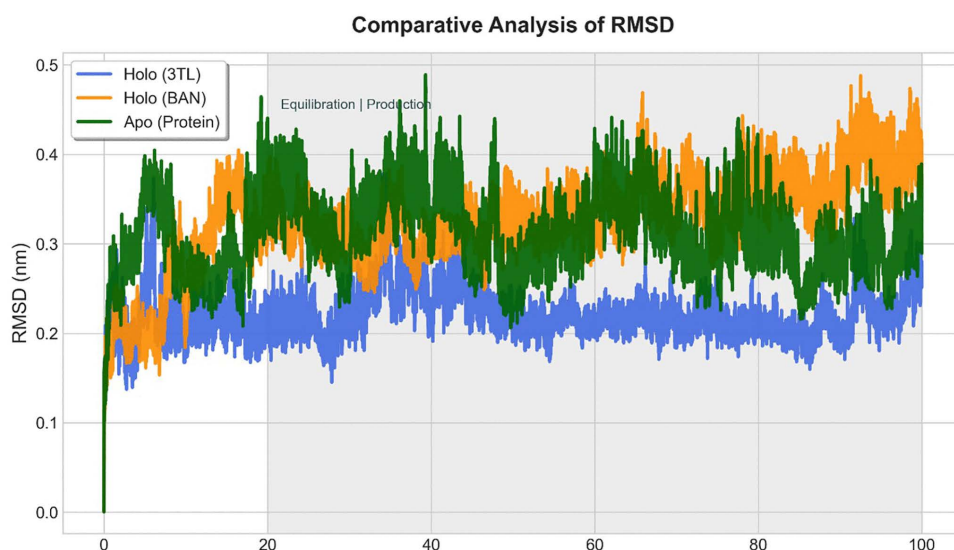


Fig 2. Comparative analysis of protein backbone RMSD. RMSD trajectories of the protein backbone for the Apo system (Apo-Protein, dark green) and the two holo complexes (Holo (3TL), royal blue; Holo (BAN), orange) over 100 ns. The shaded region denotes the production phase (after 20 ns), used for calculating convergence statistics. The Holo (3TL) system demonstrated the lowest average deviation ($\mu=0.223$ nm), indicating the highest conformational stability.

<https://doi.org/10.1371/journal.pone.0336765.g002>

Table 1. Descriptive statistics of RMSD findings.

Metric (nm)	Holo (3TL)	Holo (BAN)	Apo (Protease)
Mean	0.221	0.327	0.313
ProdMean	0.223	0.342	0.318
Max	0.378	0.488	0.489
Min	0.001	0.001	0.001
ProdStDev	0.026	0.037	0.041
SEM	0.000	0.000	0.000
StDev	0.027	0.052	0.044

<https://doi.org/10.1371/journal.pone.0336765.t001>

Holo BAN was nearly double that of the other two systems (0.215 nm), and its maximum fluctuation reaches 0.874 nm. The elevated flexibility in the Holo BAN complex confirms that the ligand induced protease instability as observed in the RMSD findings. The BAN ligand appears to destabilize critical regions of the protein.

Ligand-protein interaction energy. The total interaction energy provides a thermodynamic measure of the favorable or unfavorable binding affinity between the ligand and the protein (Fig 4). As a result, the Holo BAN complex maintained highly favorable, negative, total interaction energies throughout the production phase, settling at a mean value of -69.112 kJ/mol (Table 3). This signifies strong, persistent, and energetically favorable non-covalent interactions (electrostatic and van der Waals) between the BAN ligand and the receptor. Conversely, the Holo 3TL complex consistently displayed highly unfavorable total interaction energies, with a production mean of $+95.529$ kJ/mol. This suggests strong repulsive components, likely unfavorable electrostatic or desolvation penalties, that overwhelm any favorable van der Waals contributions. Accordingly, structurally more stable Holo 3TL system with the highly positive interaction energy indicates a thermodynamically unfavorable ligand-protein association, and the protease remains resemble to the apo system. The Holo

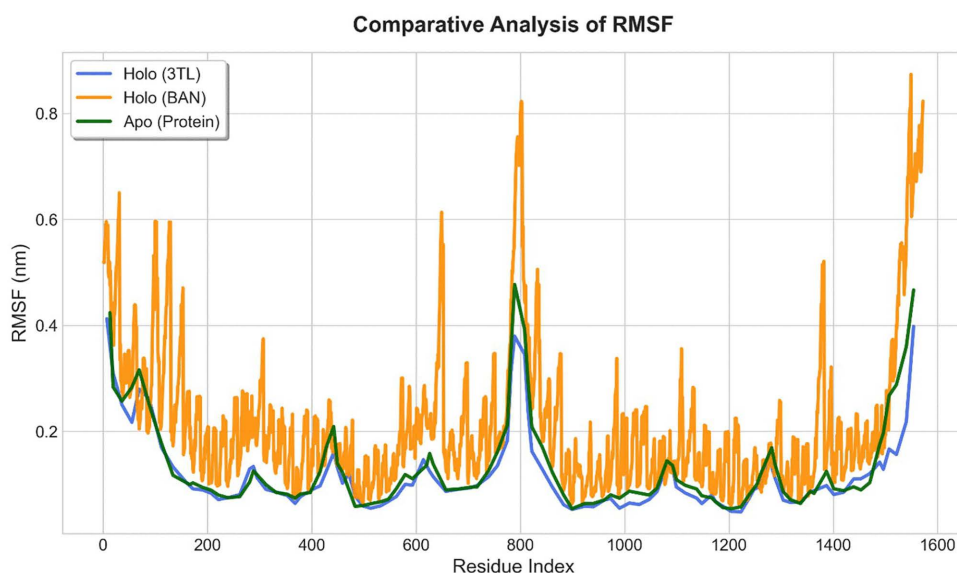


Fig 3. Comparative analysis of residue-wise RMSF. Root Mean Square Fluctuation (RMSF) per residue for the Apo system (dark green) and the Holo complexes (3TL in blue, BAN in orange). The Apo-Protein and Holo (3TL) systems share a similar, low-fluctuation profile. The Holo (BAN) complex exhibits significantly elevated flexibility (higher peaks) across several loop regions, confirming structural destabilization induced by the BAN ligand.

<https://doi.org/10.1371/journal.pone.0336765.g003>

Table 2. Descriptive statistics of RMSF findings.

Metric (nm)	Holo (3TL)	Holo (BAN)	Apo (Protease)
Mean	0.121	0.215	0.137
Max	0.412	0.874	0.477
Min	0.048	0.06	0.054
SEM	0.008	0.004	0.009
StDev	0.077	0.141	0.093

<https://doi.org/10.1371/journal.pone.0336765.t002>

BAN complex, on the other hand, is held together by significant and favorable binding interactions, highlighting stable bonding and altering flexibility of HIV-1 protease.

Solvent accessible surface area. The SASA was analyzed to track conformational changes related to solvent exposure, which is often indicative of protein compaction or unfolding. All three systems exhibited similar average SASA values, with Holo 3TL at 64.314 nm², Apo-Protein at 63.261 nm², and Holo BAN at 65.899 nm² during production (Fig 5, Table 4). The standard deviations are also tightly clustered ($\approx 1.7 - 1.8$ nm²). The data suggest that ligand binding, even by the destabilizing BAN, does not induce large-scale domain rearrangements or unfolding events that would dramatically increase or decrease the overall solvent exposure of the protein. The minor observed SASA increase in Holo BAN is consistent with the heightened residual mobility (RMSF) of external loops, which briefly expose more surface area.

Hydrogen bond analysis. To elucidate the binding stability and key interactions of the inhibitors with the HIV-1 protease, a detailed hydrogen bond analysis was performed. Hydrogen bonds are critical for the affinity and specificity of ligand-protein interactions. For the reference inhibitor 3TL, a total of 13 unique hydrogen bonds were identified between the ligand and the protease active site residues. The analysis revealed several persistent interactions, although most were transient, indicating dynamic binding. The most significant H-bond was observed between the main chain of GLY48 and the side chain of 3TL, with an occupancy of 0.78%. Another notable interaction occurred between the 3TL side chain

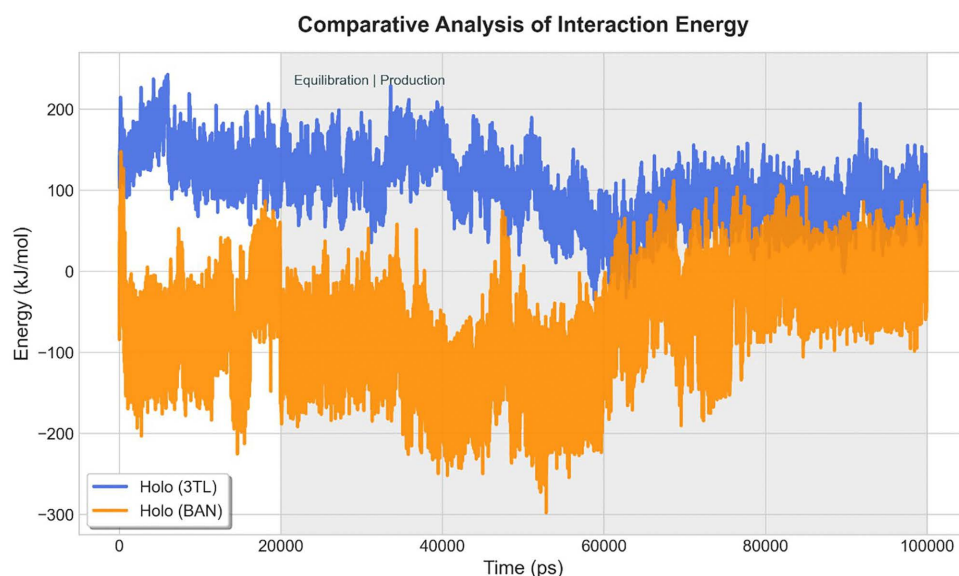


Fig 4. Comparative analysis of total ligand-protein interaction energy. Total non-bonded interaction energy (electrostatic plus van der Waals) between the protein and the bound ligands over 100 ns. This metric is only applicable to the holo systems. The Holo BAN complex displays highly favorable (negative) binding energy ($\mu = -69.112$ kJ/mol), while the Holo 3TL complex shows highly unfavorable (positive) energy ($\mu = +95.529$ kJ/mol).

<https://doi.org/10.1371/journal.pone.0336765.g004>

Table 3. Descriptive statistics of interaction energies.

Metric (kJ/mol)	Holo (3TL)	Holo (BAN)
Mean	104.641	-68.885
ProdMean	95.529	-69.112
Max	242.638	147.378
Min	-34.922	-297.954
ProdStDev	37.459	63.743
SEM	0.285	0.193
StDev	40.328	60.914

<https://doi.org/10.1371/journal.pone.0336765.t003>

and the main chain of PHE53, showing an occupancy of 0.67%. Other residues forming transient hydrogen bonds with 3TL included MET46 (0.22%), LYS55 (0.17% and 0.11%), ILE50 (0.14%), LYS45 (0.14%), and GLY49 (0.11%). The time evolution of the total number of hydrogen bonds and the occupancy of specific interactions are detailed in Fig 6.

In contrast, BAN established a more extensive and stable hydrogen bonding network, with a total of 18 unique hydrogen bonds identified (Fig 7). A remarkably persistent interaction was observed between BAN and the catalytic residue ASP25. This interaction was maintained for the majority of the simulation, demonstrating an occupancy of over 100% (165.76%), suggesting that multiple hydrogen bonds were concurrently formed between the side chains of BAN and ASP25. Furthermore, BAN formed several other stable H-bonds, including with the main chain of LEU24 (20.66%), GLY48 (11.38%), and GLY49 (8.29%). This robust network of interactions suggests a strong binding affinity of BAN for the protease.

Salt bridge analysis. Salt bridges contribute significantly to the structural stability and integrity of proteins. We analyzed the intramolecular salt bridges within the HIV-1 protease structure when complexed with each inhibitor to assess conformational stability. Several key salt bridges were monitored throughout the simulation. For the 3TL complex, the most

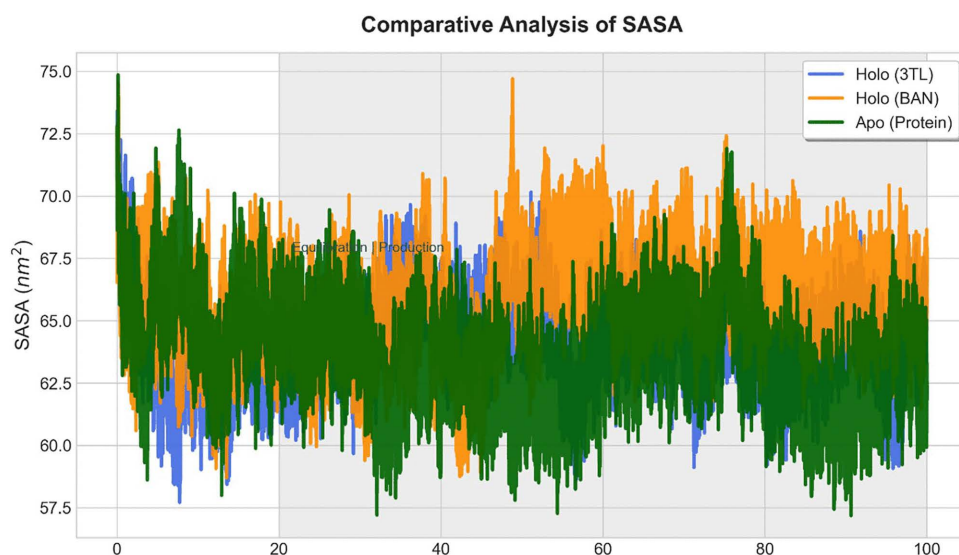


Fig 5. Comparative analysis of protein solvent accessible surface area (SASA). SASA trajectories for all three simulated systems over 100 ns. All systems show narrow distributions and similar mean SASA values (≈ 63 – 66 nm²), suggesting that ligand binding does not induce major changes in the overall global compaction or solvent exposure of the protein.

<https://doi.org/10.1371/journal.pone.0336765.g005>

Table 4. Descriptive statistics of SASA findings.

Metric (nm ²)	Holo (3TL)	Holo (BAN)	Apo (Protease)
Mean	64.158	65.794	63.651
ProdMean	64.314	65.899	63.261
Max	73.425	74.703	74.858
Min	57.712	58.698	57.18
ProdStDev	1.637	1.832	1.761
SEM	0.013	0.006	0.006
StDev	1.799	1.83	1.992

<https://doi.org/10.1371/journal.pone.0336765.t004>

stable salt bridge was formed between GLU34 and LYS20, demonstrating an occupancy of 18.66%. This was followed by an interaction between ASP30 and LYS45, which had an occupancy of 10.24%. The majority of other potential salt bridges were found to be highly transient, with occupancies below 2%. In this regard, the interaction between ASP29 and ARG87 was present for only 0.99% of the simulation time. The persistence of these specific electrostatic interactions suggests their importance in maintaining the folded architecture of the protease during the simulation (Fig 8).

As shown in Fig 9, the analysis of the BAN-protease complex revealed a notable decrease in the stability of these key intramolecular salt bridges. The occupancy of the GLU34-LYS20 interaction dropped significantly to 3.91%, and the ASP30-LYS45 interaction was present for only 2.47% of the simulation time. This reduction in salt bridge stability suggests that the binding of BAN may induce a conformational state in the protease that is distinct from the 3TL-bound form, potentially involving greater flexibility or a slight rearrangement of charged residues.

Principal component analysis. PCA was performed on the C-alpha atoms of the protease to identify the large-scale collective motions and explore the conformational landscape sampled by the enzyme when bound to the inhibitors. The analysis condenses the complex atomic fluctuations into a set of principal components (PCs), with the first few PCs describing the most significant protein motions. For the 3TL-protease complex, the first four principal components

Hydrogen Bond Analysis Summary

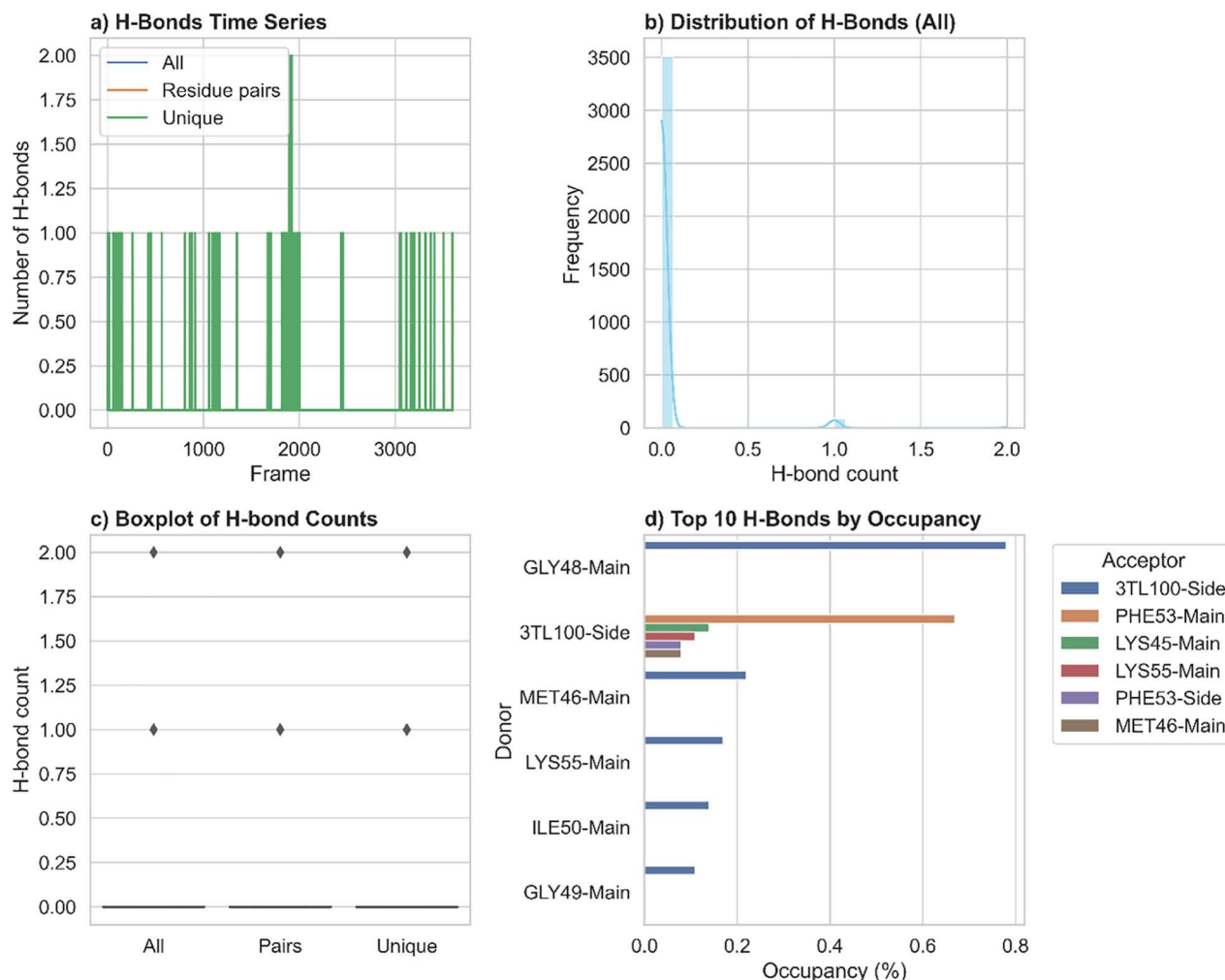


Fig 6. Hydrogen bond dynamics of the HIV-1 protease–3TL complex. Hydrogen bond occupancy and lifetime analysis between HIV-1 protease and 3TL over the simulation trajectory. The persistence of low hydrogen bonds emphasizes the low-to-medium polar interactions, which serve as a benchmark for comparison with the hit compound BAN.

<https://doi.org/10.1371/journal.pone.0336765.g006>

(PC1-PC4) accounted for 61.29% of the total variance in atomic motion observed during the simulation. PC1, representing the most dominant motion, contributed 37.02%, while PC2 contributed 10.17% of the total variance. The projection of the simulation trajectory onto the plane defined by PC1 and PC2 reveals that the protein atoms occupied a relatively defined and compact conformational space, suggesting that the binding of 3TL confers significant stability to the enzyme, restricting large-scale movements (Fig 10). The porcupine plots illustrate that the dominant motion captured by PC1 corresponds to the characteristic “flapping” motion of the protease flaps.

The PCA of the BAN-protease complex indicated a greater degree of conformational flexibility (Fig 11). The first four PCs accounted for a larger portion of the total variance (72.07%), with PC1 alone responsible for 45.70% of the motion. The increased variance captured by the initial principal components, along with a higher overall trace of the covariance

Hydrogen Bond Analysis Summary

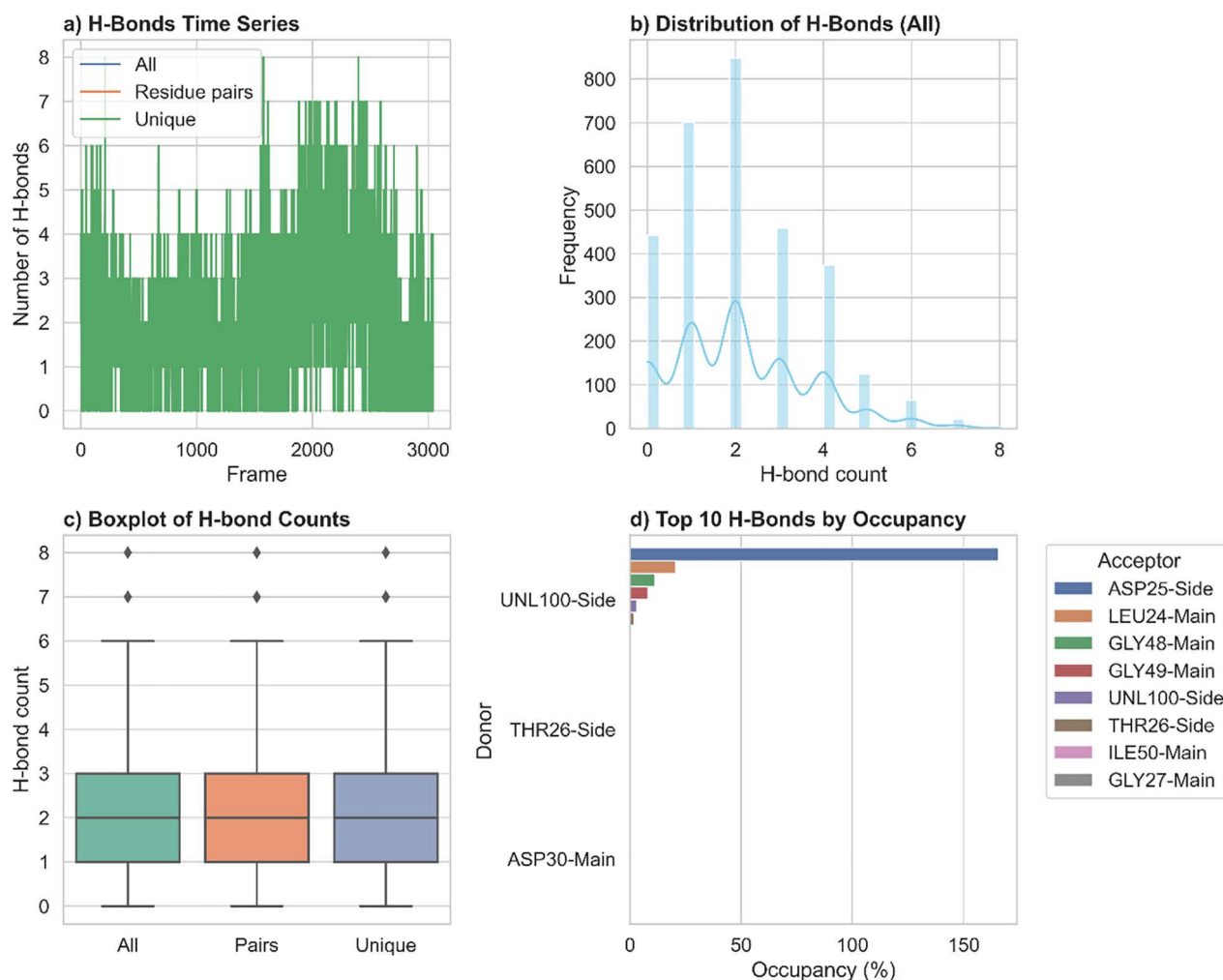


Fig 7. Binding conformation of BAN within the HIV-1 protease active site. Representative structural snapshot of the HIV-1 protease–BAN complex obtained from the converged MD simulations at 20 ns to 100 ns. The identified hit compound BAN occupies the catalytic cleft and shows close proximity to the catalytic Asp25/Asp25' dyad. The orientation suggests potential for hydrogen bonding and hydrophobic contacts, indicating its suitability as a protease inhibitor scaffold. All paired hydrogen bindings were unique. * UNL or Unknown ligand = BAN.

<https://doi.org/10.1371/journal.pone.0336765.g007>

matrix (3.49 for BAN vs. 2.15 for 3TL), suggests that the protease experiences more pronounced and coordinated motions when bound to BAN. The 2D projection plot for the BAN complex is expected to cover a larger conformational space compared to 3TL, consistent with the reduced salt bridge stability and indicative of enhanced flap dynamics. This heightened flexibility could have implications for the inhibitor's mechanism of action and residence time in the active site.

Discussion

The high-throughput pharmacophore screen of DrugBank presented BAN as a top hit matching all features of the 3TL pharmacophore. Docking predicted BAN's binding energy to be slightly better than the reference 3TL. This modest

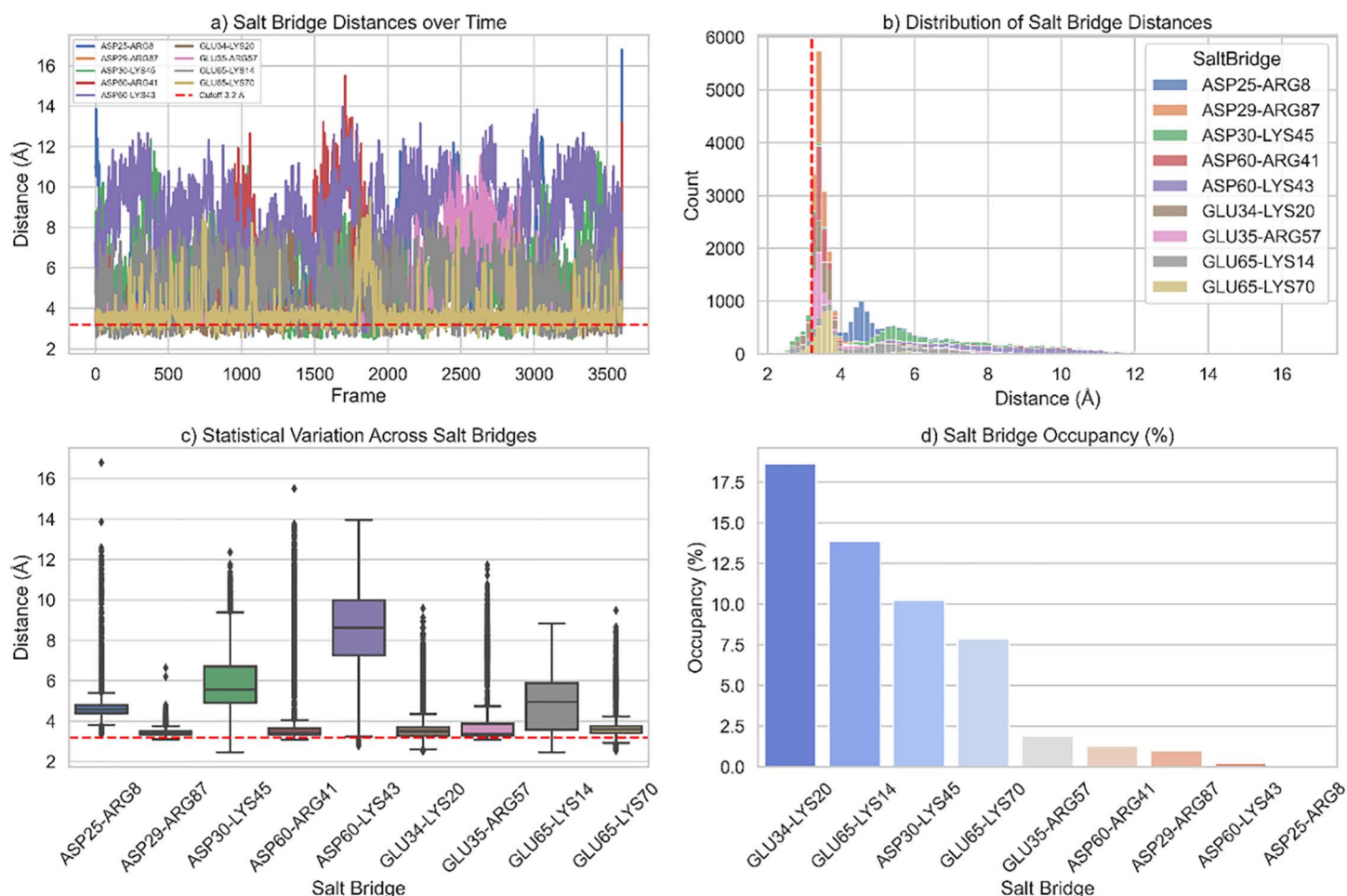


Fig 8. Salt bridge dynamics of the HIV-1 protease–3TL complex. Time-dependent profile of salt bridge interactions formed between charged residues of HIV-1 protease and the reference inhibitor 3TL during a 100-ns MD simulation. The plot demonstrates stable and persistent salt bridge contacts that contribute to structural stabilization of the protease–inhibitor complex, highlighting the role of electrostatic interactions in maintaining high-affinity binding.

<https://doi.org/10.1371/journal.pone.0336765.g008>

advantage likely arises from BAN's favorable intermolecular contacts and lower intramolecular strain. BAN's interaction energy in MD was highly favorable (mean -69.1 kJ/mol). A positive interaction energy for 3TL suggests that any favorable van der Waals contacts were offset by desolvation or repulsive electrostatics, consistent with the ligand rigidifying the protease without forming compensating polar bonds. In contrast, BAN formed persistently favorable interactions that stabilized the complex. These observations mirror prior reports, stating potent HIV-1 PR ligands often achieve large negative free energies primarily via polar and hydrophobic contacts [18]. Accordingly, a quinazoline inhibitor (CID-2135609) showed very stable binding and maintained key contacts with Asp25 and other residues during MD simulation [18]. Similarly, BAN's strong binding energy correlates with its robust hydrogen-bond network.

However, BAN's binding conferred greater structural flexibility to the protease than 3TL. The backbone RMSD of the BAN-bound complex stabilized around 0.34 nm, substantially higher than 3TL's ~ 0.22 nm. Especially, Holo 3TL stabilized very quickly and maintained minimal fluctuation, indicating a highly stable conformation under MD simulation. This behavior is akin to that reported for high-affinity inhibitors in which CID-2135609-bound PR showed minimal backbone deviation

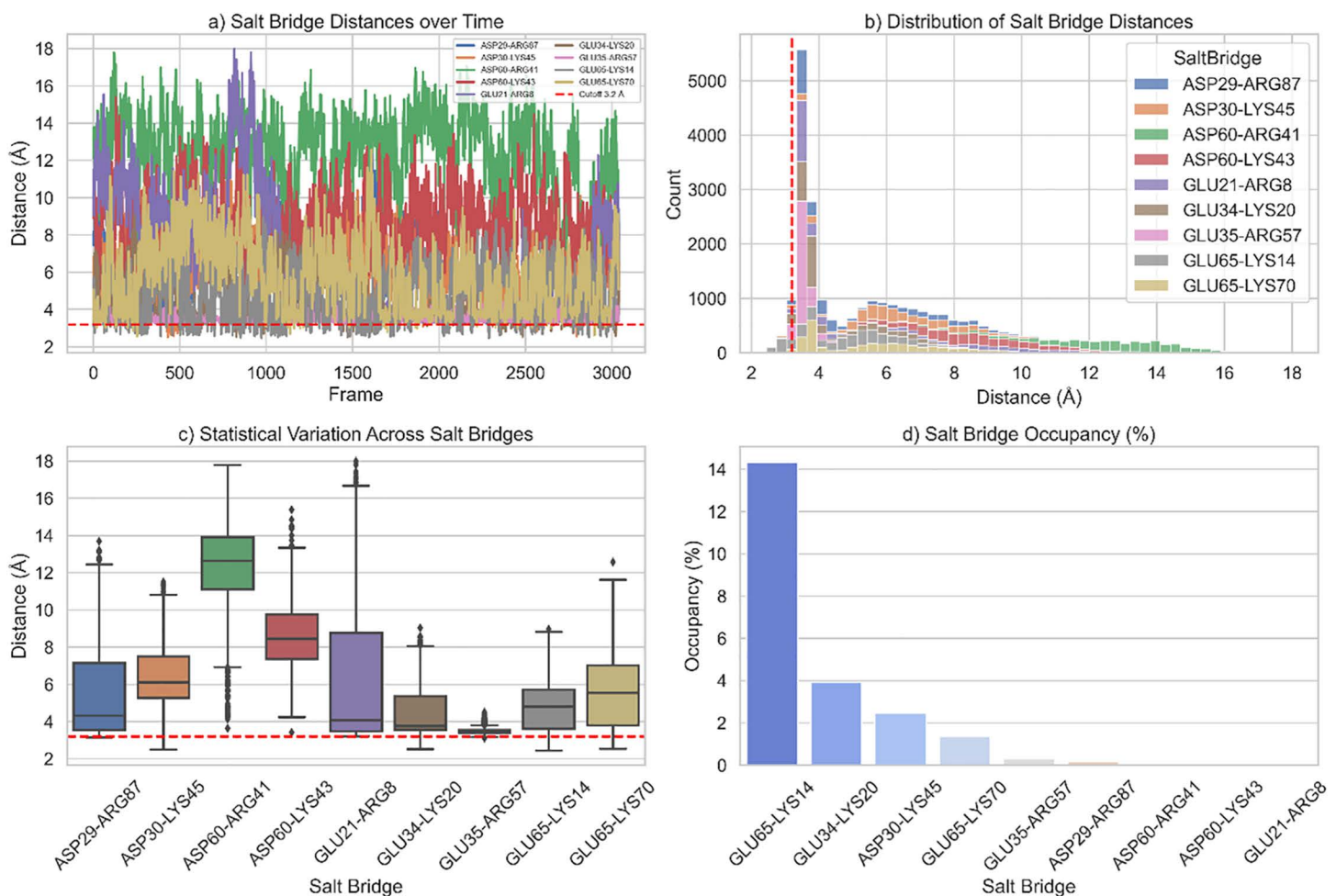


Fig 9. Salt bridge dynamics of the HIV-1 protease-BAN complex. Time evolution of salt bridge interactions in the BAN-bound protease. The occupancy and stability of electrostatic contacts suggest differences in charge stabilization compared to the 3TL reference complex. The data indicate that BAN binding induces a distinct electrostatic interaction pattern, potentially contributing to an altered dynamic profile of the protease.

<https://doi.org/10.1371/journal.pone.0336765.g009>

and conserved secondary structure [18]. By contrast, BAN induced larger RMSD oscillations, suggesting that its bound state allows the protein to deviate more from the starting structure. Residue RMSF analysis confirmed this. Accordingly, the BAN complex exhibits broad flexibility increases across loop and flap regions, residues 50–250, 750–950, and a peak near 1500. The average RMSF of BAN-bound PR was nearly double that of 3TL-bound. Such enhanced mobility is consistent with fewer stabilizing intraprotein constraints. In agreement, the Asp25 catalytic flaps likely sample wider conformational space when BAN is bound. In contrast, 3TL's RMSF profile closely matched the apo enzyme, indicating 3TL rigidifies the enzyme much like CID-2135609 and other potent PIs do [16].

Despite BAN's induced flexibility, all three systems had similar global SASA (63–66 nm²), implying no large-scale unfolding. The slight SASA increase in the BAN complex aligns with its elevated loop mobility. Protruding loops modestly expose more surface area without collapsing the structure. Particularly, previous work observed only minor SASA changes upon ligand binding for stable PR complexes [18]. Thus, BAN's effect is subtle at the tertiary scale.

A key distinction between BAN and 3TL is their hydrogen-bonding patterns. Over 100 ns MD, 3TL formed only 13 transient H-bonds with PR (highest occupancies 0.7–0.8%), mainly to Gly48 and Phe53. In contrast, BAN established 18

Principal Component Analysis (PCA) Summary

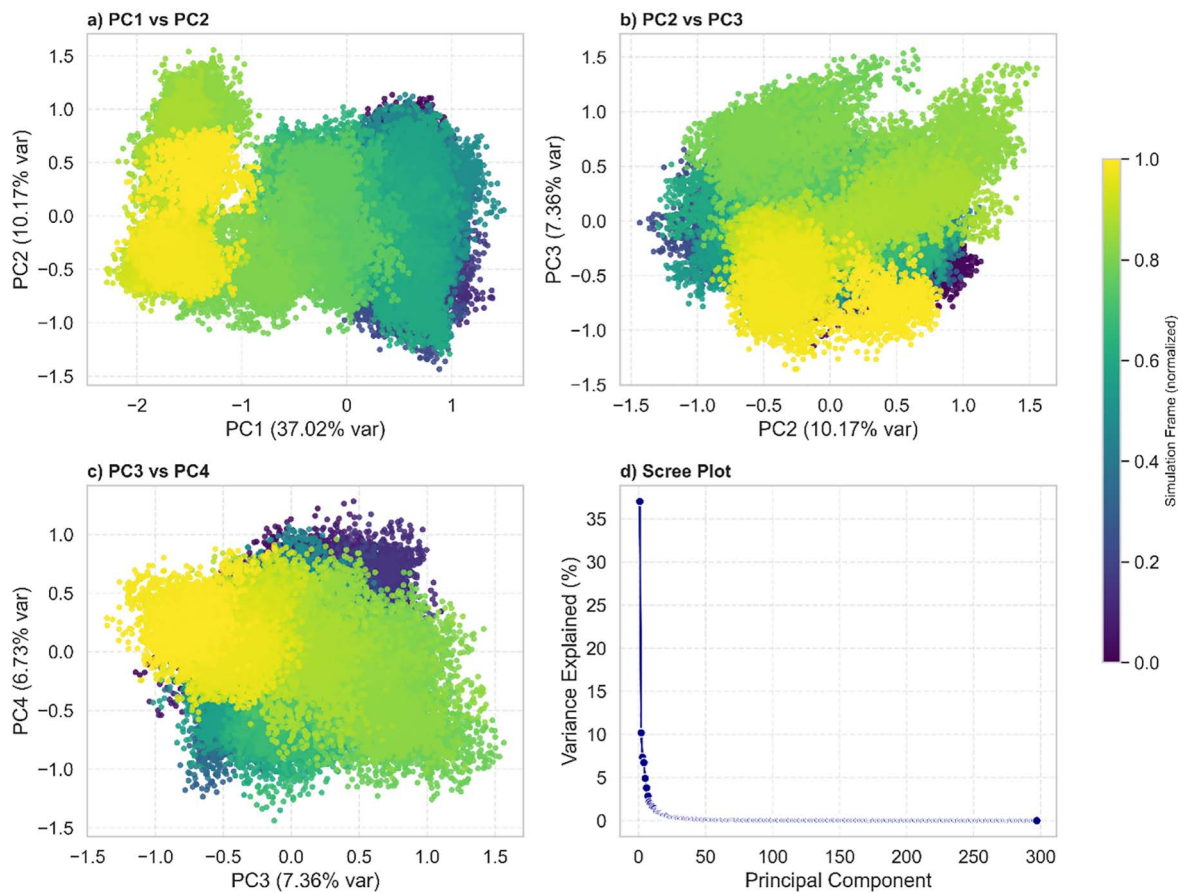


Fig 10. Principal component analysis of the HIV-1 protease-3TL complex. PCA plots describing the conformational landscape of the protease in complex with 3TL. (A, B, and C) Projection of trajectory frames along PC1 vs. PC2, PC2 vs. PC3, and PC3 vs. PC4 showing limited conformational fluctuations. (D) Eigenvalue distribution reflecting constrained collective motions. Free energy landscapes indicating well-defined minima corresponding to stable states of the protease in the presence of 3TL. These findings suggest that 3TL binding restricts protease flexibility more effectively than BAN.

<https://doi.org/10.1371/journal.pone.0336765.g010>

H-bonds, including a highly persistent one with Asp25 (>100% occupancy, indicating multiple concurrent bonds) and stable interactions with Leu24 (20.7%), Gly48 (11.4%), and Gly49 (8.3%). This dense H-bond network likely underlies BAN's favorable interaction energy. These findings are in agreement with other studies. In this regard, residues Asp25, Gly27–30, and Ile50 often mediate PR inhibitor binding via H-bonds or van der Waals forces [37–39]. For example, CID-2135609 engaged Asp25 and Gly49 as key contacts [18]. The persistent Asp25 bonding by BAN is particularly encouraging, as this catalytic residue is a critical anchor in effective inhibitors. In contrast, 3TL's lack of strong Asp25 bonding suggests it relies more on hydrophobic packing than on polar anchoring. Overall, BAN's richer polar contacts hint at strong affinity despite the protein's concomitant gain in flexibility.

Interestingly, BAN binding weakened several stabilizing intraprotein salt bridges. In the 3TL complex, the GLU34–LYS20 salt bridge (18.7% occupancy) and ASP30–LYS45 (10.2%) were the most persistent electrostatic contacts. These salt bridges likely help clamp PR's folds. BAN reduced their occupancies drastically (GLU34–LYS20 to 3.9%;

Principal Component Analysis (PCA) Summary

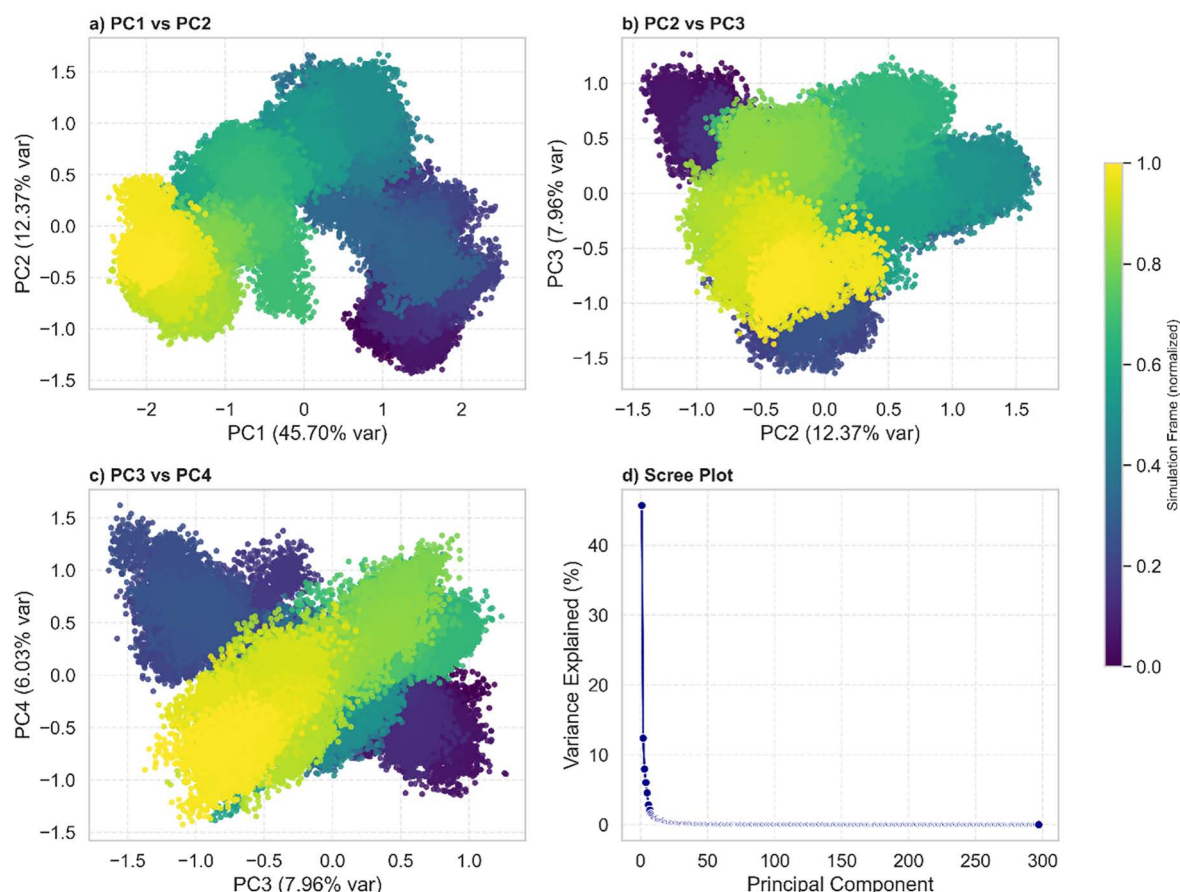


Fig 11. PCA of the HIV-1 protease–BAN complex. Essential dynamics of the BAN-bound protease represented by PCA. (A, B, and C) Projection of the MD trajectory along the first two principal components (PC1 vs. PC2), showing conformational sampling. (D) Distribution of eigenvalues, indicating the dominance of low-frequency motions. Two-dimensional free energy landscapes constructed from PC1 and PC2, highlighting preferred conformational states of the protease in the presence of BAN. Together, the analysis reveals the conformational adaptability of the enzyme upon BAN binding.

<https://doi.org/10.1371/journal.pone.0336765.g011>

ASP30–LYS45 to 2.5%). The loss of these interactions is consistent with the higher RMSF and PCA variance observed for BAN-bound PR. To our knowledge, prior computational HIV-1 PR studies have seldom detailed salt-bridge dynamics; nonetheless, it is plausible that attenuating these electrostatic anchors contributes to the increased flap motion seen with BAN. Overall, BAN appears to induce a distinct conformational ensemble, trading intraprotein stability for stronger ligand contacts.

PCA analysis provided further insight. Accordingly, the first four PCs of 3TL-bound PR captured 61.3% of variance (PC1 37.0%), whereas BAN-bound PR captured 72.1% (PC1 45.7%). The larger cumulative variance and trace, 3.49 vs 2.15, indicate more collective motion under BAN. The dominant mode corresponds to the flap “open-closed” motion. This suggests that BAN, despite binding tightly, permits more pronounced flap mobility than 3TL. The wider spread of trajectory points in the BAN PCs reinforces that BAN samples a broader conformational space. Such enhanced dynamics could affect inhibitor efficacy or residence time. Overall, 3TL binding confines the protease into a narrow, stable energy basin, while BAN induces a more adaptable state with deeper local minima, likely aided by its extensive H-bond network.

Taken together, this study indicates that BAN, HONH-BENZYLMALONYL-L-ALANYLGLYCINE-P-NITROANILIDE, is capable of binding HIV-1 PR with affinity comparable to 3TL, via both hydrophobic and strong polar interactions. However, BAN's mechanism differs. It sustains the protease-ligand association by forming additional hydrogen bonds at Asp25 while simultaneously loosening intraprotein salt bridges and increasing overall flexibility. This contrasts with 3TL and other high-affinity PIs, which tend to "lock" PR into a rigid bound state. Thus, BAN represents a novel scaffold whose inhibition profile may be enthalpically driven. Future work should evaluate whether BAN's dynamic binding leads to different inhibition kinetics or resistance profiles. The combined literature and simulation analyses underscore the importance of integrating multiple computational metrics to fully characterize protease-inhibitor complexes.

Conclusions

Here, a DrugBank-derived compound, BAN, identified as a promising HIV-1 protease inhibitor through structure-based pharmacophore screening, docking, and MD simulation. Docking predicted BAN binds the protease with energy comparable to the reference inhibitor 3TL. Over 100 ns MD, BAN formed a robust hydrogen-bond network, including a highly persistent Asp25 interaction, and achieved a favorable total interaction energy. However, BAN also induced notably higher backbone and side-chain fluctuations in the enzyme compared to 3TL. BAN binding disrupted key intramolecular salt bridges and enlarged the conformational ensemble. In contrast, 3TL stabilized the protease in a rigid, low-mobility state with few lasting H-bonds. These findings suggest BAN's binding is energetically favorable but dynamically distinct, balancing strong polar contacts with induced flexibility. The similar SASA across systems implies no unfolding, but the elevated mobility under BAN may influence inhibitor potency or resistance. Altogether, BAN emerges as a novel scaffold worthy of experimental validation. Its distinct binding signature, strong enthalpic interactions coupled with dynamic protein response, highlights a new chemotype for HIV-1 PR inhibition. Future optimization of BAN's structure could yield high-affinity inhibitors that exploit its potent Asp25 interactions while enhancing structural rigidity.

Supporting information

S1 Table. Statistical results of analyzes. Data includes the information of GROMACS MD simulation analysis of Holo (3TL, BAN) vs Apo (pro) with statistical tests.
(TXT)

S2 Checklist. Inclusivity in global research questionnaire. The filled checklist of the inclusivity in global research, which aims to improve transparency in the reporting of research performed outside of researchers' own country or community and ensures that PLOS publications reporting global research adhere to high standards for research ethics and authorship.
(DOCX)

Author contributions

Conceptualization: Amjed Majid Rashid Alrashedi.

Data curation: Ali Sultan Khazaal Nazal, Amjed Majid Rashid Alrashedi.

Formal analysis: Ali Sultan Khazaal Nazal, Mustafa Abdulkadhim Hussein Ali Rabeea.

Investigation: Ali Sultan Khazaal Nazal, Lamia Abdultef Risan Al-Iessa.

Methodology: Amjed Majid Rashid Alrashedi.

Project administration: Amjed Majid Rashid Alrashedi.

Resources: Lamia Abdultef Risan Al-Iessa, Mustafa Abdulkadhim Hussein Ali Rabeea.

Software: Ali Sultan Khazaal Nazal, Amjed Majid Rashid Alrashedi, Lamia Abdultef Risan Al-Iessa.

Supervision: Amjed Majid Rashid Alrashedi.

Validation: Amjed Majid Rashid Alrashedi, Lamia Abdultef Risan Al-Iessa, Mustafa Abdulkadhim Hussein Ali Rabeea.

Visualization: Amjed Majid Rashid Alrashedi.

Writing – original draft: Ali Sultan Khazaal Nazal, Amjed Majid Rashid Alrashedi, Lamia Abdultef Risan Al-Iessa, Mustafa Abdulkadhim Hussein Ali Rabeea.

Writing – review & editing: Ali Sultan Khazaal Nazal, Amjed Majid Rashid Alrashedi, Lamia Abdultef Risan Al-Iessa, Mustafa Abdulkadhim Hussein Ali Rabeea.

References

1. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 2015;1–2:19–25. <https://doi.org/10.1016/j.softx.2015.06.001>
2. Galaxy Community. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update. *Nucleic Acids Res*. 2022;50(W1):W345–51. <https://doi.org/10.1093/nar/gkac247> PMID: 35446428
3. Agniswamy J, Kneller DW, Brothers R, Wang Y-F, Harrison RW, Weber IT. Highly drug-resistant HIV-1 protease mutant PRS17 shows enhanced binding to substrate analogues. *ACS Omega*. 2019;4(5):8707–19. <https://doi.org/10.1021/acsomega.9b00683> PMID: 31172041
4. Arrigoni R, Santacroce L, Ballini A, Palese LL. AI-aided search for new HIV-1 protease ligands. *Biomolecules*. 2023;13(5):858. <https://doi.org/10.3390/biom13050858> PMID: 37238727
5. Askari FS, Ebrahimi M, Parhiz J, Hassanpour M, Mohebbi A, Mirshafiey A. Digging for the discovery of SARS-CoV-2 nsp12 inhibitors: a pharmacophore-based and molecular dynamics simulation study. *Future Virol*. 2022. <https://doi.org/10.2217/fvl-2022-0054> PMID: 35983350
6. Askari FS, Mohebbi A. The dynamic states of hepatitis B virus capsid monomers under the impact of different class of capsid-assembly modulators. *Sci Rep*. 2025;15(1):32731. <https://doi.org/10.1038/s41598-025-18339-6> PMID: 40993170
7. Baassi M, Moussaoui M, Soufi H, Rajkhowa S, Sharma A, Sinha S, et al. Towards designing of a potential new HIV-1 protease inhibitor using QSAR study in combination with molecular docking and molecular dynamics simulations. *PLoS One*. 2023;18(4):e0284539. <https://doi.org/10.1371/journal.pone.0284539> PMID: 37079533
8. Baei B, Askari P, Askari FS, Kiani SJ, Mohebbi A. Pharmacophore modeling and QSAR analysis of anti-HBV flavonols. *PLoS One*. 2025;20(1):e0316765. <https://doi.org/10.1371/journal.pone.0316765> PMID: 39804828
9. Blankenberg D, Coraor N, Von Kuster G, Taylor J, Nekrutenko A. Integrating diverse databases into an unified analysis framework: a Galaxy approach. *Database*. 2011. <https://doi.org/10.1093/database/bar011>
10. Case DA, Aktulga HM, Belfon K, Cerutti DS, Cisneros GA, Cruzeiro VWD, et al. AmberTools. *J Chem Inf Model*. 2023;63:6183–91. https://doi.org/10.1021/ACS.JCIM.3C01153/ASSET/IMAGES/LARGE/CI3C01153_0002.JPEG
11. Case DA, Cerutti DS, Cruzeiro VWD, Darden TA, Duke RE, Ghazimirsaeed M, et al. Recent developments in amber biomolecular simulations. *J Chem Inf Model*. 2025;65(15):7835–43. <https://doi.org/10.1021/acs.jcim.5c01063> PMID: 40728386
12. Case DA, Cheatham TE 3rd, Darden T, Gohlke H, Luo R, Merz KM Jr, et al. The Amber biomolecular simulation programs. *J Comput Chem*. 2005;26(16):1668–88. <https://doi.org/10.1002/jcc.20290> PMID: 16200636
13. Chuntakaruk H, Hengphasatporn K, Shigeta Y, Aonbangkhen C, Lee VS, Khotavivattana T, et al. FMO-guided design of darunavir analogs as HIV-1 protease inhibitors. *Sci Rep*. 2024;14(1):3639. <https://doi.org/10.1038/s41598-024-53940-1> PMID: 38351065
14. Dakshinamoorthy A, Asmita A, Senapati S. Comprehending the structure, dynamics, and mechanism of action of drug-resistant HIV protease. *ACS Omega*. 2023;8(11):9748–63. <https://doi.org/10.1021/acsomega.2c08279> PMID: 36969469
15. Das D, Koh Y, Tojo Y, Ghosh AK, Mitsuya H. Prediction of potency of protease inhibitors using free energy simulations with polarizable quantum mechanics-based ligand charges and a hybrid water model. *J Chem Inf Model*. 2009;49(12):2851–62. <https://doi.org/10.1021/ci900320p> PMID: 19928916
16. Deganutti G, Prischi F, Reynolds CA. Supervised molecular dynamics for exploring the druggability of the SARS-CoV-2 spike protein. *J Comput Aided Mol Des*. 2021;35(2):195–207. <https://doi.org/10.1007/s10822-020-00356-4> PMID: 33103220
17. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. *J Chem Inf Model*. 2021;61:3891–8. https://doi.org/10.1021/ACS.JCIM.1C00203/SUPPL_FILE/C11C00203_SI_002.ZIP
18. Falls Z, Fine J, Chopra G, Samudrala R. Accurate prediction of inhibitor binding to HIV-1 protease using CANDOCK. *Front Chem*. 2022;9:775513. <https://doi.org/10.3389/fchem.2021.775513> PMID: 35111726
19. Martin SA, Cane PA, Pillay D, Mbisa JL. Coevolved multidrug-resistant HIV-1 protease and reverse transcriptase influences integrase drug susceptibility and replication fitness. *Pathogens*. 2021;10(9):1070. <https://doi.org/10.3390/pathogens10091070> PMID: 34578103

20. Mirarab A, Mohebbi A, Moradi A, Javid N, Vakili MA, Tabarraei A. Frequent pUL27 variations in HIV-infected patients. *Intervirology*. 2016;59(5–6):262–6. <https://doi.org/10.1159/000471484> PMID: 28402975
21. Mohebbi A. Ligand-based 3D pharmacophore modeling, virtual screening, and molecular dynamic simulation of potential smoothened inhibitors. *J Mol Model*. 2023;29. <https://doi.org/10.1007/s00894-023-05532-5>
22. Mohebbi A, Askari FS, Sammak AS, Ebrahimi M, Najafimemar Z. Druggability of cavity pockets within SARS-CoV-2 spike glycoprotein and pharmacophore-based drug discovery. *Future Virol*. 2021a. <https://doi.org/10.2217/fvl-2020-0394> PMID: 34099962
23. Mohebbi A, Askari FS, Sammak AS, Ebrahimi M, Najafimemar Z. Druggability of cavity pockets within SARS-CoV-2 spike glycoprotein and pharmacophore-based drug discovery. *Future Virol*. 2021b. <https://doi.org/10.2217/fvl-2020-0394> PMID: 34099962
24. Mohebbi A, Mirarab A, Shaddel R, Shafaei Fallah M, Memarian A. Molecular dynamic simulation and docking of cyclophilin A mutants with its potential inhibitors. *J Clin Basic Res*. 2021c;5(2):26–41. <https://doi.org/10.52547/jcbr.5.2.26>
25. Mohebbi A, Mohammadi S, Memarian A. Prediction of HBF-0259 interactions with hepatitis B virus receptors and surface antigen secretory factors. *Virusdisease*. 2016;27(3):234–41. <https://doi.org/10.1007/s13337-016-0333-9> PMID: 28466034
26. Mohebbi A, Nabavi SPT, Naderi M, Sharifian K, Behnezhad F, Mohebbi M, et al. Computer-aided drug repurposing & discovery for Hepatitis B capsid protein. *In Silico Pharmacol*. 2025;13(1):35. <https://doi.org/10.1007/s40203-025-00314-8> PMID: 40018383
27. Nabavi SPT, Askari FS, Askari P, Mohebbi A. Molecular dynamic simulation of Cyclophilin A in complex with Sanglifehrin A. 2024. <https://doi.org/10.21203/RS.3.RS-5005208/V1>
28. Namthabhad S, Appl EM-IJR. Molecular docking of HIV-1 protease using alkaloids from *Tinospora cordifolia*. *Int J Res Appl*. 2014;1:12–6. [https://doi.org/10.17812/ijra.1.1\(3\)2014](https://doi.org/10.17812/ijra.1.1(3)2014)
29. Okafor SN, Angsantikul P, Ahmed H. Discovery of novel HIV protease inhibitors using modern computational techniques. *Int J Mol Sci*. 2022;23(20):12149. <https://doi.org/10.3390/ijms232012149> PMID: 36293006
30. Perryman AL, Zhang Q, Soutter HH, Rosenfeld R, McRee DE, Olson AJ, et al. Fragment-based screen against HIV protease. *Chem Biol Drug Des*. 2010;75(3):257–68. <https://doi.org/10.1111/j.1747-0285.2009.00943.x> PMID: 20659109
31. Rahman MM, Saha T, Islam KJ, Suman RH, Biswas S, Rahat EU, et al. Virtual screening, molecular dynamics and structure-activity relationship studies to identify potent approved drugs for Covid-19 treatment. *J Biomol Struct Dyn*. 2021;39(16):6231–41. <https://doi.org/10.1080/07391102.2020.1794974> PMID: 32692306
32. Rehan Ahmad S, Zeyaulah M, AlShahrani AM, Mohieldin A, Alam MS, Altijani AAG. Structure-based identification and molecular characterization of CID-2135609 as a potent small molecule modulator of HIV-1 protease. *J Cell Biochem*. 2025;126(8):e70058. <https://doi.org/10.1002/jcb.70058> PMID: 40792456
33. Sadybekov AV, Katritch V. Computational approaches streamlining drug discovery. *Nature*. 2023;616(7958):673–85. <https://doi.org/10.1038/s41586-023-05905-z> PMID: 37100941
34. Śledź P, Caflisch A. Protein structure-based drug design: from docking to molecular dynamics. *Curr Opin Struct Biol*. 2018;48:93–102. <https://doi.org/10.1016/j.sbi.2017.10.010> PMID: 29149726
35. Sousa da Silva AW, Vranken WF. ACPYPE - AnteChamber PYthon Parser interfacE. *BMC Res Notes*. 2012;5:367. <https://doi.org/10.1186/1756-0500-5-367> PMID: 22824207
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31(2):455–61. <https://doi.org/10.1002/jcc.21334> PMID: 19499576
37. Venkatachalam S, Muralidharan N, Pandian R, Sayed Y, Gromiha MM. Integrative computational approaches for understanding drug resistance in HIV-1 protease subtype C. *Viruses*. 2025;17(6):850. <https://doi.org/10.3390/v17060850> PMID: 40573441
38. Volarath P, Harrison RW, Weber IT. Structure based drug design for HIV protease: from molecular modeling to cheminformatics. *Curr Top Med Chem*. 2007;7(10):1030–8. <https://doi.org/10.2174/156802607780906744> PMID: 17508936
39. Weber IT, Kneller DW, Wong-Sam A. Highly resistant HIV-1 proteases and strategies for their inhibition. *Future Med Chem*. 2015;7(8):1023–38. <https://doi.org/10.4155/fmc.15.44> PMID: 26062399