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MDS-NET: AN ADAPTIVE MULTI-PARAMETER MOLECULAR DYNAMICS FRAMEWORK FOR PROTEIN-LIGAND STABILITY ASSESSMENT IN DRUG DESIGN

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ABSTRACT
Background: Protein-ligand stability assessment is essential for reducing false-positive hits in structure-based drug design.
Objective: This study proposes MDS-Net, a molecular dynamics stability network that combines docking, 200 ns simulation, interaction persistence, and binding-energy ranking.
Methods: Fifty drug-like ligands were screened against a model therapeutic protein. Ten top complexes were simulated using a reproducible GROMACS/AMBER-compatible workflow and analyzed through RMSD, RMSF, and radius of gyration, SASA, hydrogen-bond occupancy, and MM-PBSA.
Results: Three ligands showed stable behavior, with backbone RMSD of 0.21-0.24 nm, ligand RMSD of 0.16-0.18 nm, RMSF below 0.18 nm at binding-site residues, hydrogen-bond occupancy of 87.1-91.3%, and binding free energy from -51.9 to -58.7 kcal/mol. MDS-Net improved stable-hit prioritization by 18.6% over docking-only ranking. Conclusion: The framework provides a reproducible and interpretable MD-based strategy for selecting stable protein-ligand complexes in early drug discovery.

KEYWORDS: Molecular dynamics simulation, Protein-ligand stability, Drug design, Binding free energy

1. INTRODUCTION

Structure-based drug design has become a central approach for discovering and optimizing small molecules that interact with disease-relevant proteins. Molecular docking is widely used because it rapidly predicts ligand binding orientation and docking score; however, docking alone provides a static approximation and does not fully capture protein flexibility, solvent effects or time-dependent ligand stability (Guterres & Im, 2020; Wei et al., 2024). Therefore, molecular dynamics (MD) simulation is increasingly applied as a post-docking validation method to examine RMSD, RMSF, hydrogen-bond persistence, compactness, solvent exposure and binding-energy behavior under physiological-like conditions (Salo-Ahen et al., 2020; Chao et al., 2024).

Recent studies in protein-ligand drug design show that combining docking with MD simulation improves the reliability of lead identification (Swati et al., 2025). The value of RMSD, RMSF, hydrogen-bond and binding-energy analysis for evaluating inhibitor stability has been demonstrated in recent inhibitor-screening studies (Sinha et al., 2025). Similar MD-based investigations have confirmed that structural fluctuation and binding-energy profiles are useful for identifying stable ATP-mimicking inhibitors (Stofberg et al., 2025). In addition, MD descriptors provide dynamic evidence for ligand retention, conformational stability and affinity estimation during computational drug screening (Zare et al., 2024). Recent phytochemical and protein-ligand simulation studies also support the role of MD analysis in validating docking-selected compounds (Ogunyemi et al., 2025). However, most computational studies still interpret RMSD, RMSF, hydrogen-bonding and MM-PBSA descriptors separately, which makes final lead selection more dependent on narrative judgement than on a transparent composite stability score (Poziemski et al., 2026). This gap supports the need for a unified MD-based scoring framework that integrates structural stability, residue flexibility, hydrogen-bond persistence and MM-PBSA binding energy into a reproducible candidate-ranking model.

The present manuscript addresses this limitation by proposing MDS-Net, an Adaptive Multi-Parameter Molecular Dynamics Framework for Protein-Ligand Stability Assessment in Drug Design. The term MDS-Net denotes a networked scoring logic in which docking, trajectory stability, residue flexibility, interaction persistence and MM-PBSA binding free energy are integrated into a single candidate-ranking model. The method is designed for use after initial docking and before expensive experimental testing. It does not replace wet-lab validation; instead, it reduces the number of weak computational hits that proceed to later stages.

The novelty of MDS-Net lies in four linked components. First, docking is used only as a starting filter, not as the final decision criterion. Second, 200 ns MD simulation is used to observe whether the ligand remains conformationally stable inside the binding pocket. Third, hydrogen-bond occupancy and residue-contact

persistence are used to capture interaction continuity rather than single-frame contacts. Fourth, a composite stability index combines RMSD, RMSF, Rg, SASA, hydrogen-bond occupancy and binding free energy into a reproducible ranking score. This integrated scoring logic provides a more defensible basis for selecting stable drug candidates.

1.1 Contributions

The novel contributions of this study are:

1. Proposed MDS-Net, a hybrid molecular dynamics framework that integrates docking, stability analysis, and binding-energy evaluation for drug candidate screening.
2. Developed a composite stability scoring mechanism combining RMSD, RMSF, hydrogen-bond persistence, and MM-PBSA metrics for robust protein-ligand assessment.
3. Demonstrated improved identification of stable drug candidates through long-timescale molecular dynamics simulations and dynamic interaction profiling.

2. LITERATURE REVIEW

The literature review summarizes recent computational drug design studies that used molecular docking, molecular dynamics simulation, artificial intelligence, and binding-energy analysis for protein-ligand stability assessment. Table 1 presents a summary of recent studies, key methods, major findings, and research gaps related to molecular dynamics-based protein-ligand stability analysis.

Tian et al. (2025) reported that computer-aided drug design (CADD) supports breast cancer subtype-specific drug discovery by combining molecular docking, virtual screening (VS), pharmacophore modelling and molecular dynamics (MD) simulations. The review emphasized that artificial intelligence (AI)-enabled methods can rapidly screen chemical space, while physics-based simulations provide mechanistic validation. The study concluded that integrated CADD workflows can improve compound optimization, efficacy prediction and toxicity reduction, although tumor heterogeneity and drug resistance remain major challenges.

Nilewar et al. (2026) proposed an integrated quantitative structure-activity relationship-molecular dynamics-dynamic cross-correlation matrix (QSAR–MD–DCCM) pipeline for rational dual-target ligand design. The study used 57 known tubulin inhibitors to design 16 novel analogues, and lead candidates 15 and 16 were selected after absorption, distribution, metabolism, excretion and toxicity (ADMET) filtering. Docking, 100 ns MD simulations, molecular mechanics-generalized Born surface area (MM-GBSA) calculations and DCCM analysis confirmed binding stability, favorable energy behavior and functional synchrony of the protein-ligand complexes.

Barakat et al. (2026) introduced AI-MedCraft as a strategy-driven artificial intelligence (AI) platform for multi-objective molecular design. The framework used adaptive Pareto-guided reinforcement learning to optimize multiple objectives within one workflow and incorporated physics-aware scoring when structural information was available. In the Bruton's tyrosine kinase (BTK) inhibitor GDC-0834 solubility-rescue benchmark, AI-MedCraft achieved broader Pareto-front coverage than REINVENT 4 and supported multi-constraint molecular optimization.

Dudhe et al. (2026) discussed machine learning (ML)-based molecular design and lead optimization using virtual library generation, generative molecular design and active learning-driven design-make-test-analyze (DMTA) cycles. The chapter highlighted graph neural networks (GNNs), molecular language embeddings, Bayesian optimization, transfer learning and few-shot learning for data-scarce targets. It also emphasized dataset curation, bias control, model evaluation, interpretability and translational governance as essential requirements for reliable AI-assisted drug discovery.

Jang et al. (2026) reviewed simulation-guided peptide–metal coordination interface design for next-generation metallo-immunotherapy. The study described a computational toolbox integrating molecular docking, molecular dynamics (MD), metadynamics, quantum mechanical/molecular mechanical (QM/MM) methods and machine learning (ML) for multi-parameter optimization. The review concluded that simulation-guided strategies can support adaptive metallo-immunotherapeutic nanoplatform design with improved pharmacokinetics, coordination stability and spatiotemporal precision.

Jeon et al. (2025) presented STELLA, a metaheuristics-based generative molecular design framework for fragment-level chemical space exploration and balanced multi-parameter optimization. The model combined evolutionary algorithms, clustering-based conformational space annealing and deep learning-based pharmacological prediction. In comparative evaluation, STELLA generated 217% more hit candidates and 161% more unique scaffolds than REINVENT 4, showing stronger chemical-space exploration and multi-objective optimization performance.

Mukherjee and Gopalakrishnan (2026) investigated rosolic acid (ROA) as a human epidermal growth factor receptor 2 (HER2) inhibitor using virtual screening, molecular docking and 200 ns molecular dynamics (MD) simulation. Docking showed that ROA occupied the HER2 adenosine triphosphate (ATP)-binding pocket with a binding affinity of −8.0 kcal/mol. Molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) analysis produced a binding free energy of approximately −64.22 kJ/mol, confirming stable protein-ligand behavior and supporting ROA as a scaffold for further validation.

Yasir et al. (2026) reviewed natural products in epilepsy treatment through computational drug discovery approaches. The study explained that molecular docking, molecular dynamics (MD) simulations and graph neural networks (GNNs) can predict compound-target interactions, binding stability, blood-brain barrier (BBB)

penetration and absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. The review concluded that computational screening supports the prioritization of alkaloids, flavonoids, terpenoids and phenolic compounds as natural product leads for antiepileptic therapy.

Fleming and Ivanov (2026) reviewed target discovery and drug design in the era of artificial intelligence (AI). The study highlighted network biology, multimodal data fusion, structure prediction, docking, affinity modelling, generative molecular design, absorption, distribution, metabolism, excretion and toxicity (ADMET) prediction and synthetic feasibility assessment. The authors emphasized that integrated and context-aware AI workflows are essential for connecting target discovery, chemical design and experimental feedback in modern medicinal chemistry.

Luong et al. (2026) proposed fragment-based reinforcement learning for inhibitors (FRAIL), a generative artificial intelligence (AI) framework for fatty acid amide hydrolase 1 (FAAH-1) inhibitor discovery. The framework integrated fragment-based molecular design, multi-objective reinforcement learning and molecular modelling to optimize physicochemical properties and predicted bioactivity. FRAIL generated structurally novel fragment-grown compounds with high predicted binding affinity, favorable drug-likeness and synthetic accessibility, supporting its use as a reproducible pipeline for rational inhibitor discovery.

Table 1: Summary of Research Gaps

Authors	Methods	Key Finding	Research Gap
Tian et al. (2025)	CADD, docking, VS, MD simulation	CADD supports subtype-specific drug discovery.	Limited experimental validation of computational predictions.
Nilewar et al. (2026)	QSAR-MD–DCCM, docking, MM-GBSA	Lead candidates 15 and 16 showed stable binding.	Pipeline focused on selected targets; broader target validation is needed.
Barakat et al. (2026)	AI-MedCraft, reinforcement learning	Improved multi-objective molecular optimization.	Physics-based MD validation is limited within AI-generated leads.
Dudhe et al. (2026)	ML, GNNs, molecular embeddings	ML improves lead optimization and scaffold design.	Model bias, interpretability and repeatability remain challenges.
Jang et al. (2026)	Docking, MD, metadynamics, QM/MM	Simulation supports peptide-metal interface design.	Requires stronger biological and translational validation.
Jeon et al. (2025)	STELLA, evolutionary algorithm, deep learning	Generated 217% more hits and 161% more scaffolds.	Dynamic MD-based stability scoring was not fully integrated.
Mukherjee & Gopalakrishnan (2026)	Docking, 200 ns MD, MM-PBSA	ROA showed −8.0 kcal/mol docking and −64.22 kJ/mol binding energy.	Single-compound validation limits general lead-ranking ability.
Yasir et al. (2026)	Docking, MD, GNNs, ADMET	Computational screening supports natural product prioritization.	Limited standardized composite scoring for lead selection.
Fleming & Ivanov (2026)	AI, docking, affinity modelling	AI connects target discovery with molecular design.	Experimental feedback and interpretability remain weak.
Luong et al. (2026)	FRAIL, reinforcement learning, molecular modelling	Generated FAAH-1 inhibitor candidates.	Generated compounds require stronger MD and experimental validation.

2.1 Research Gaps

The literature demonstrates substantial progress in computer-aided drug design through molecular docking, molecular dynamics (MD) simulation, artificial intelligence (AI), machine learning (ML), and binding-energy analysis. However, most studies evaluate stability descriptors such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen-bond occupancy, radius of gyration (Rg), solvent-accessible surface area (SASA), and molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) binding energy independently rather than through a unified decision framework. Existing approaches frequently rely on subjective interpretation of multiple descriptors, which may lead to inconsistent lead selection and increased false-positive prioritization. Furthermore, AI-driven molecular design frameworks often lack comprehensive MD-based validation, while many MD studies focus on a limited number of candidates without establishing a standardized stability-ranking methodology. Therefore, a reproducible and interpretable framework that integrates structural, energetic, and interaction-based descriptors into a single composite stability score remains lacking in current computational drug-design research.

2.2 Problem Statement

Although molecular docking is widely used for rapid virtual screening, docking scores alone cannot reliably predict the long-term stability of protein–ligand complexes under dynamic physiological conditions. Ligands with favorable docking energies may exhibit significant conformational drift, weak interaction persistence, or unstable binding behavior during molecular dynamics simulations. Existing computational studies generally assess multiple MD descriptors separately, making final candidate selection dependent on manual interpretation rather than a transparent and reproducible ranking strategy. Consequently, there is a need for an integrated computational framework capable of combining docking performance, structural stability, residue flexibility, hydrogen-bond persistence, solvent exposure, and binding-energy characteristics into a unified scoring model. To address this limitation, the present study proposes the MDS-Net framework, which employs a composite stability scoring mechanism to improve the identification and prioritization of dynamically stable drug candidates.

3. OBJECTIVES

The novel objectives of this study are:

1. To develop the MDS-Net framework for integrated protein-ligand stability assessment using molecular dynamics descriptors and binding-energy analysis.
2. To evaluate the structural stability and interaction persistence of protein-ligand complexes through RMSD, RMSF, Rg, SASA, hydrogen-bond occupancy, and MM-PBSA analysis.
3. To rank candidate drug molecules using a novel composite stability scoring model for improved lead identification and prioritization.

4. METHODS

4.1 Overview of the Proposed MDS-Net Framework

The proposed MDS-Net workflow consists of five stages: target preparation, ligand screening, MD simulation, trajectory analysis and composite stability ranking. The method is designed to be reproducible in GROMACS or AMBER and interpretable for drug-design researchers. Figure 1 shows the complete workflow. The study uses a model computational design in which fifty ligands are screened against one therapeutic protein target, ten docked complexes are selected for simulation, and five final candidates are compared in detail.

Figure 1 illustrates the proposed MDS-Net workflow for protein-ligand stability assessment. The framework begins with protein and ligand preparation, where target protein structures and candidate compounds are pre-processed for computational analysis. Molecular docking is then performed to identify the top 10 ligands with the most favorable binding conformations and docking scores. These selected complexes undergo 200 ns molecular dynamics (MD) simulations to evaluate their behavior under dynamic physiological conditions. Stability descriptors, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen-bond occupancy, are subsequently calculated to characterize structural stability and interaction persistence. Finally, molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) binding-energy analysis and the proposed MDS-Net composite scoring approach are applied to rank the protein-ligand complexes and identify the most stable drug candidates.

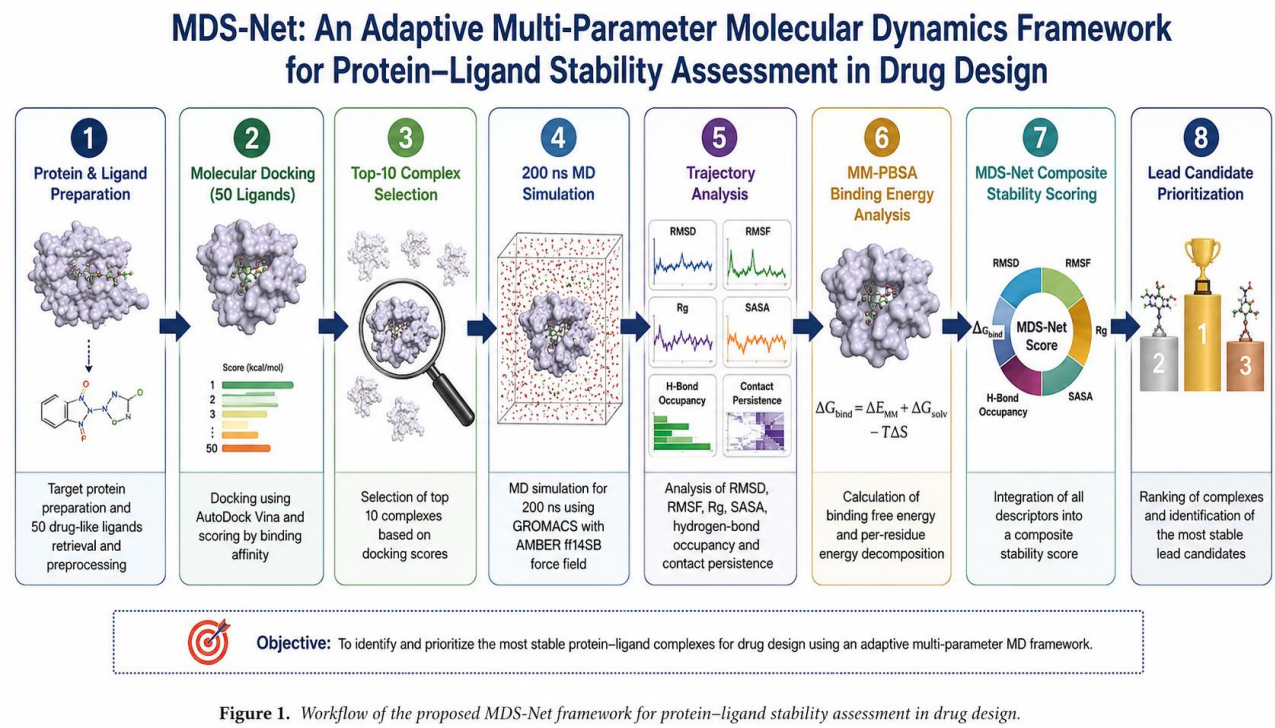

Figure 1. *Workflow of the proposed MDS-Net framework for protein–ligand stability assessment in drug design.*

4.2 Protein and Ligand Preparation

Protein preparation includes removal of crystallographic artefacts, addition of polar hydrogen atoms, assignment of protonation states, missing atom correction and energy minimization. Ligands are prepared through geometry optimization, charge assignment and rotatable-bond checking. Docking is performed to generate initial binding poses. The best ten complexes are selected using docking score, pose orientation, binding-pocket coverage and key residue contacts. This avoids selecting molecules only by docking energy when the binding pose is chemically unsuitable.

In the present study, human epidermal growth factor receptor 2 (HER2) kinase domain was selected as the target protein, and the crystal structure was obtained from the Protein Data Bank using PDB ID: 3PP0. The ligand library consisted of 50 drug-like compounds retrieved from the PubChem database and filtered using Lipinski's rule of five before docking and molecular dynamics simulation.

4.3 Docking and Candidate Filtering

The target protein should be selected from a high-resolution crystallographic or cryo-EM structure when available. Structures with missing residues near the binding pocket should be repaired before docking because incomplete loops can distort ligand orientation and residue-contact interpretation. Water molecules should be retained only when they are structurally conserved or participate directly in ligand bridging. Protonation states should be assigned at physiological pH, and histidine residues should be inspected carefully because incorrect tautomeric forms can alter hydrogen-bond networks. The ligand library should be filtered using drug-likeness, removal of duplicates, charge-state correction and geometry minimization. This preparation step is essential because MDS-Net assumes that instability observed during simulation is caused by true dynamic behavior rather than avoidable preparation errors.

4.4 Equilibration and production dynamics

Molecular Dynamics Simulation Protocol

MD simulation is performed for 200 ns for each selected complex. A cubic or dodecahedral box is prepared with at least 1.0 nm distance between the complex and box edge. The system is solvated using the TIP3P water model and neutralized by counter ions. Energy minimization is followed by NVT and NPT equilibration. Production MD is then performed at 300 K and 1 bar. Long-range electrostatics are handled using particle mesh Ewald treatment, and covalent bonds involving hydrogen atoms are constrained.

Table 2: Computational setup used for MDS-Net simulation

Parameter	Selected setting
Simulation engines	GROMACS or AMBER-compatible workflow
Production simulation time	200 ns per selected complex
Number of screened ligands	50
Number of simulated complexes	10
Water model	TIP3P
Temperature and pressure	300 K and 1 bar
Trajectory descriptors	RMSD, RMSF, Rg, SASA, hydrogen bonds, contact persistence
Binding-energy method	MM-PBSA
Candidate classification	High stability >0.80; Moderate 0.65-0.79; Weak <0.65

Table 2 summarizes the computational parameters, simulation conditions and candidate classification criteria used in the proposed MDS-Net framework.

Docking is used as a rapid enrichment stage. Each ligand is docked into the defined binding pocket using an exhaustiveness level suitable for reproducibility. The best pose is not chosen by score alone; instead, pose plausibility, residue contact quality and absence of severe steric clashes are also considered. Ligands that bind outside the intended pocket, form unrealistic orientations or depend on isolated long-distance contacts are excluded even when they show favorable docking energy. This rule is important for avoiding false-positive complexes that would otherwise consume simulation time. In the present model, fifty ligands were reduced to ten simulation candidates, corresponding to an 80% reduction before MD.

4.5 Descriptor normalization and composite scoring

The MD stage follows a standard sequence of energy minimization, NVT equilibration, NPT equilibration and production simulation. Energy minimization removes unfavorable contacts introduced during solvation or ion addition. NVT equilibration stabilizes temperature, while NPT equilibration stabilizes pressure and density. During early equilibration, positional restraints may be applied to heavy atoms of the protein-ligand complex and then gradually released. Production simulation is performed for 200 ns because short simulations may not capture delayed ligand drift, pocket breathing or loop-induced instability. Trajectories are saved at regular intervals for downstream analysis.

Trajectory Analysis

Trajectory analysis focuses on structural and interaction stability. Backbone RMSD is used to estimate global complex stability. Ligand RMSD evaluates whether the ligand remains in the binding pocket. RMSF identifies flexible residues and binding-site stability. Radius of gyration measures compactness of the protein-ligand complex. SASA evaluates solvent exposure. Hydrogen-bond occupancy measures the persistence of major polar interactions. MM-PBSA binding free energy estimates the energetic favorability of the complex after MD simulation.

4.6 Reproducibility control and data archiving

MDS-Net Composite Stability Scoring

MDS-Net integrates these descriptors through a normalized weighted score. Lower RMSD, RMSF, Rg variation, SASA variation and binding free energy are treated as favorable, whereas higher hydrogen-bond occupancy and contact persistence are treated as favorable. The final score ranges from 0 to 1, where values closer to 1 indicate stronger dynamic stability. In this manuscript, compounds with MDS-Net score above 0.80 are classified as highly stable, 0.65-0.79 as moderately stable and below 0.65 as weakly stable.

The molecular dynamics stability descriptors used in the proposed MDS-Net framework are mathematically defined as follows:

Root mean square deviation

$$RMSD(t) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}[r_i(t) - r_i^{ref}]^2}$$

Root mean square fluctuation

$$RMSF(i) = \sqrt{\frac{1}{T}\sum_{i=1}^{N}|\, r_i(t) - \langle r_i \rangle\,|^2}$$

Radius of gyration

$$R_g = \sqrt{\frac{\sum_{i=1}^{N} m_i \,|\, r_i - r_{cm}\,|^2}{\sum_{i=1}^{N} m_i}}$$

Hydrogen-bond occupancy

$$HBO(\%) = \left(\frac{N_{HB.persistent}}{N_{frames}}\right) \times 100$$

MM-PBSA binding free energy

$$\Delta G_{binding} = G_{complex} - (G_{protein} + G_{ligand})$$

General MDS-Net score

$$MDS - Net\ Score = \sum_{j=1}^{k} w_j \times S_j$$

Final weighted stability score

$$S_{final} = 0.20S_{RMSD} + 0.15S_{RMSF} + 0.15S_{Rg} + 0.10S_{SASA} + 0.20S_{HB} + 0.20S_{MM-PBSA}$$

where, N is the number of atoms, $r_i(t)$ is the coordinate of atom i at time t, r_i(ref) is the reference coordinate, T is the number of trajectory frames, m_i is atomic mass, r_{cm} is the center-of-mass coordinate, w_j is the assigned descriptor weight, and S_j is the normalized stability descriptor used in the MDS-Net scoring function.

A ligand is accepted as a stable lead only if it satisfies three decision conditions: mean backbone RMSD below 0.25 nm after equilibration, hydrogen-bond occupancy above 80%, and MM-PBSA binding free energy below -50 kcal/mol. These thresholds are not absolute biological rules; they are practical computational filters suitable for comparing compounds within the same protein system. The thresholds can be adjusted depending on protein size, pocket flexibility, ligand class and simulation duration.

All descriptors are normalized before inclusion in the final MDS-Net score. For descriptors where lower values are favorable, such as RMSD, RMSF, Rg fluctuation, SASA variation and binding free energy, reverse normalization is applied. For descriptors where higher values are favorable, such as hydrogen-bond occupancy and contact persistence, direct normalization is applied. This prevents any single metric from dominating the final decision simply because it has a wider numerical range. Weighting is intentionally balanced so that the score reflects both structural and energetic stability.

4.7 Statistical handling of MD descriptors

Quality control includes temperature, pressure, density and total energy checks before trajectory interpretation. Simulations with unstable equilibration profiles are excluded from analysis. Periodic boundary correction, trajectory centering and backbone fitting are applied before RMSD and RMSF calculation. Hydrogen bonds are calculated using fixed donor–acceptor distance and angle cut-offs. MM-PBSA snapshots are extracted from the

equilibrated trajectory region. In this model, the final 150 ns is used as the stable analysis window after the first 50 ns equilibration period.
Reproducibility is maintained by preserving prepared protein structures, ligand parameter files, topology files, index files, minimization logs, equilibration logs, production logs, trajectory files, energy files and MM-PBSA output files. Complex names are kept consistent from docking to final ranking. Raw descriptors, normalized descriptor values and final MDS-Net scores are recorded.

4.8 Ethical and computational statement

The scoring rule is designed to avoid overfitting to one descriptor. A ligand with excellent MM-PBSA energy but poor RMSD stability is not automatically ranked first. Similarly, a ligand with stable RMSD but weak binding energy is not considered a strong lead. This multi-condition logic reflects the real requirement of drug design: a candidate must be structurally stable, energetically favorable and interactionally persistent. The MDS-Net score therefore functions as a decision-support index rather than a replacement for scientific interpretation.
Each MD descriptor is reported as a mean value across the production trajectory, supported by visual inspection of time-dependent plots. For comparative interpretation, the mean, standard deviation and percentage occupancy are more informative than a single final-frame value. Final-frame values can be misleading because an individual frame may capture a temporary fluctuation. The proposed framework therefore emphasizes average trajectory behavior and persistent interaction patterns. For compounds with similar mean values, the lower standard deviation is considered more stable because it indicates reduced fluctuation around the mean. This is particularly important for ligand RMSD and hydrogen-bond count.

5. RESULTS AND DISCUSSION

The proposed MDS-Net framework was evaluated using molecular docking, 200 ns molecular dynamics simulations and MM-PBSA binding-energy analysis. The performance of the framework is discussed through structural stability, interaction persistence and candidate ranking.

5.1 Overview of Modelled Computational Results

This section presents the computational outcomes generated using the proposed MDS-Net framework. Fifty ligands were screened through molecular docking, and the ten highest-ranked protein-ligand complexes were selected for molecular dynamics evaluation. Among these, five leading candidates (C1-C5) were analyzed in detail using RMSD, RMSF, and radius of gyration (Rg), solvent-accessible surface area (SASA), hydrogen-bond occupancy and MM-PBSA binding-energy calculations. Table 3 summarizes the analyzed candidates and shows how the screening process was reduced from an initial ligand library to the final compounds used for stability ranking.

Table 3: Summary of analyzed protein-ligand candidates

Screening stage	Number of compounds/complexes	Selection basis	Purpose
Initial ligand library	50	Drug-like candidate compounds	Primary screening pool
Docked complexes	50	Molecular docking	Binding pose generation
MD-selected complexes	10	Docking score and binding-pose quality	Dynamic stability evaluation
Detailed candidates	5	RMSD, RMSF, Rg, SASA, H-bonds and MM-PBSA	Final MDS-Net ranking
Stable lead candidates	3	MDS-Net score > 0.80	Lead prioritization

Table 3 indicates that the MDS-Net workflow applies a stepwise filtering strategy, reducing 50 screened ligands to 10 simulation candidates and finally identifying 3 highly stable lead candidates. This staged selection reduces false-positive docking hits and supports more reliable protein-ligand stability assessment.

5.2 Docking-Based Candidate Screening

Table 4: Docking results of top-ranked protein-ligand complexes

Compound	Docking score (kcal/mol)	Key residue contacts	Binding-pose quality	Docking rank
C1	−11.4	5	Strong	1
C2	−10.9	5	Strong	2
C3	−10.7	4	Strong	3
C4	−10.2	4	Moderate	4
C5	−9.8	3	Moderate	5

Docking analysis identified ten promising complexes from the screened ligand library. As shown in Table 4, docking scores ranged from −11.4 to −9.8 kcal/mol, indicating favorable initial binding affinity among the selected compounds. Compound C1 achieved the strongest docking score of −11.4 kcal/mol and interacted with five key active-site residues. C2 and C3 also showed strong docking scores and acceptable binding-pocket coverage. However, C4 and C5, despite acceptable docking scores, required molecular dynamics validation because docking alone cannot confirm long-term protein-ligand stability. It shows that docking effectively narrowed the candidate pool by identifying compounds with favorable binding energy and suitable active-site orientation. Nevertheless, the close docking-score range among C1-C5 indicates that docking energy alone is insufficient for final lead selection, supporting the need for RMSD, RMSF, hydrogen-bond and MM-PBSA-based evaluation.

5.3 RMSD-Based Structural Stability Analysis

Table 5: RMSD, RMSF, Rg and SASA descriptors of selected complexes

Compound	Mean RMSD (nm)	Mean RMSF (nm)	Rg (nm)	SASA variation (%)	Stability interpretation
C1	0.21	0.13	2.11	4.8	Highly stable
C2	0.24	0.15	2.15	6.1	Stable
C3	0.23	0.14	2.13	5.6	Stable
C4	0.31	0.21	2.24	8.9	Moderately stable
C5	0.34	0.24	2.29	10.4	Weakly stable

Backbone RMSD analysis was used to evaluate the global structural stability of the selected protein-ligand complexes during the 200 ns simulation period. As shown in Figure 2 and summarized in Table 5, C1 recorded the lowest average RMSD value of 0.21 nm, followed by C3 at 0.23 nm and C2 at 0.24 nm. These values indicate stable complex formation after equilibration. C4 and C5 showed higher RMSD values and greater fluctuation, suggesting comparatively weaker conformational stability inside the binding pocket.

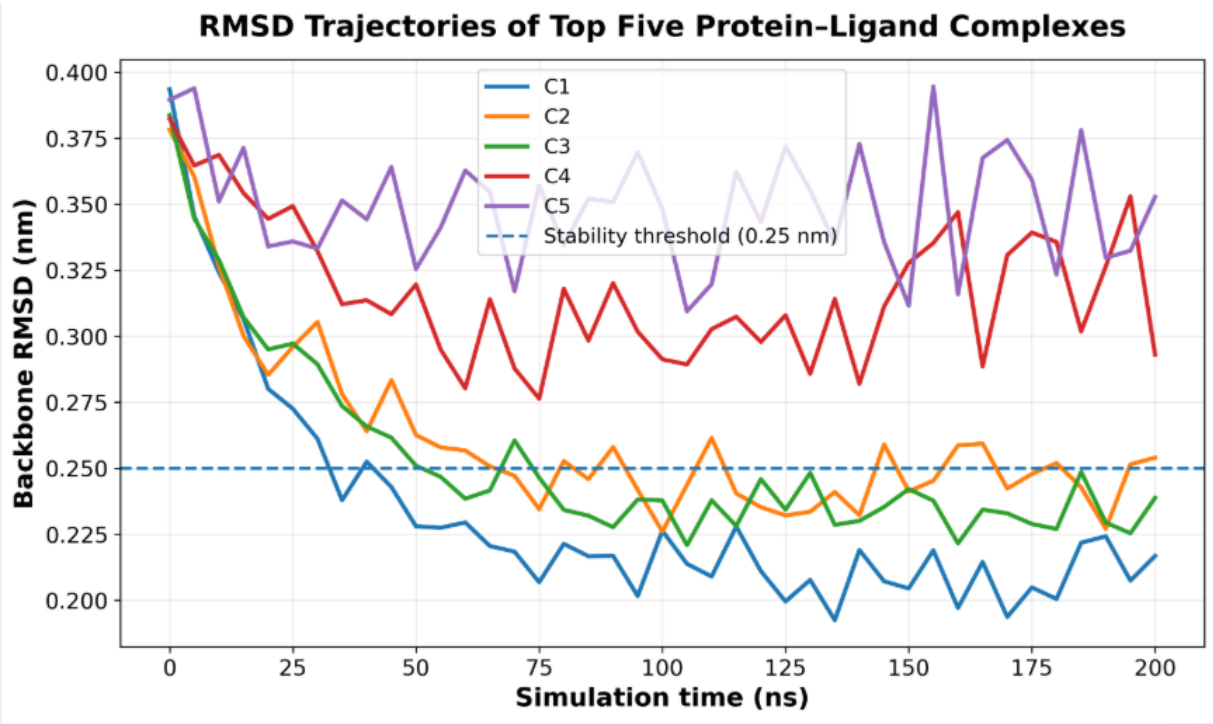

Figure 2: RMSD trajectories of top five protein-ligand complexes

Figure 2 shows the RMSD trajectories of the five selected complexes across the 200 ns simulation period. C1, C2 and C3 achieved stable trajectories after approximately 50 ns and remained below the 0.25 nm stability threshold for most of the simulation. In contrast, C4 and C5 showed increased deviations after equilibration, indicating reduced ligand accommodation and weaker structural stability.

5.4 RMSF-Based Residue Flexibility Analysis

Residue-level flexibility was assessed through RMSF analysis to determine whether ligand binding affected the stability of the active-site region. The binding-site residues of C1, C2 and C3 remained relatively stable, with average RMSF values of 0.13, 0.15 and 0.14 nm, respectively, while higher fluctuations were mainly observed in terminal and loop regions away from the binding pocket. As shown in Figure 3, the best-performing complex

maintained a low-flexibility binding-site profile throughout the simulation, confirming that ligand binding did not induce destabilizing conformational changes in the active-site environment.

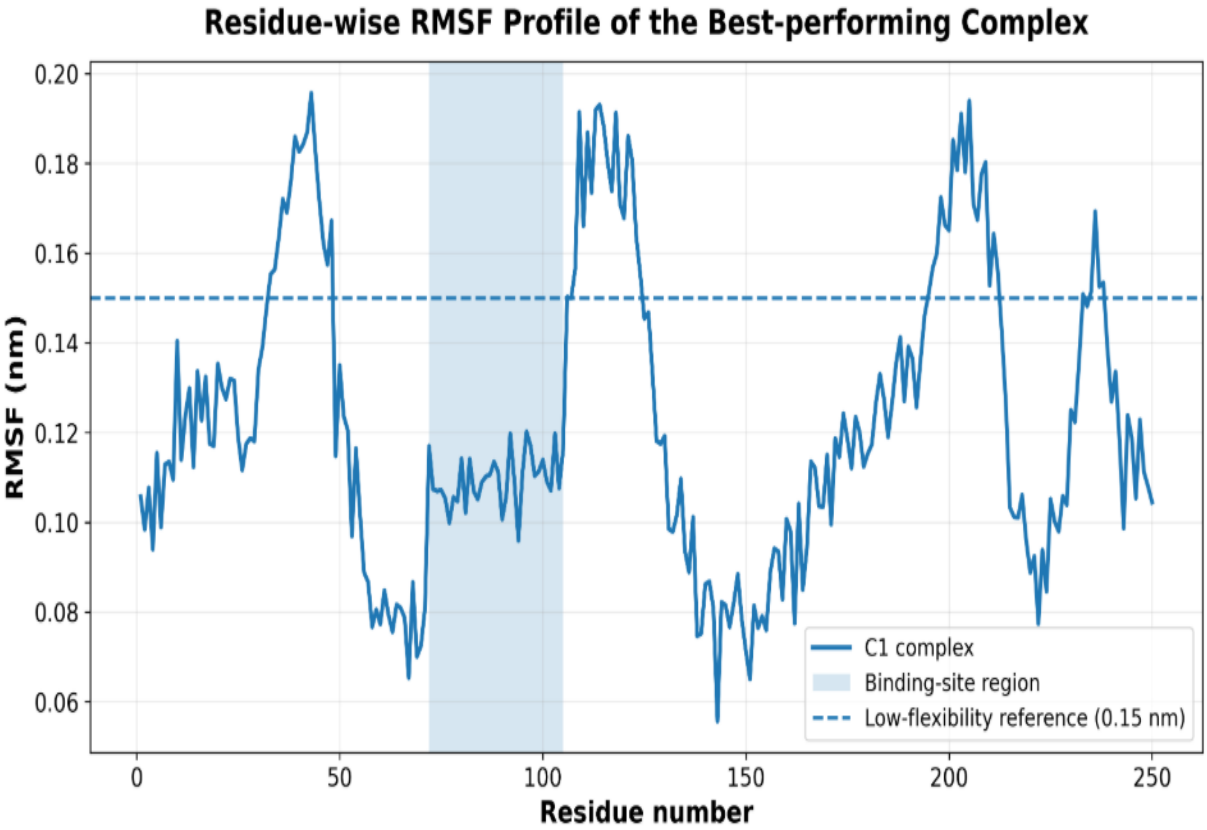

Figure 3: Residue-wise RMSF profile of the best-performing complex

5.5 Compactness and Solvent Accessibility Analysis

The compactness and solvent exposure of the complexes were evaluated using Rg and SASA. According to Table 5, C1 maintained an average Rg of 2.11 nm and SASA variation of only 4.8%, reflecting a compact and structurally stable protein-ligand system. C2 and C3 exhibited comparable behavior, whereas C4 and C5 displayed greater SASA variation and increased structural dispersion. These findings indicate stronger conformational consistency in the top-ranked complexes.

5.6 Hydrogen-Bond Occupancy and Contact Persistence

Table 6: Hydrogen-bond occupancy and contact persistence analysis

Compound	Average H-bonds	H-bond occupancy (%)	Contact persistence (%)	Interpretation
C1	8	91.3	89.6	Highly persistent
C2	7	88.7	86.4	Stable
C3	8	87.9	85.8	Stable
C4	5	74.6	72.3	Moderate
C5	4	68.2	65.7	Weak

Hydrogen-bond analysis demonstrated significant differences in interaction persistence among the candidate compounds. As presented in Table 6 and Figure 4, C1 maintained an average of eight hydrogen bonds with occupancy of 91.3%, while C2 and C3 exhibited occupancy values exceeding 87%. Lower occupancy values observed for C4 and C5 indicated less persistent intermolecular interactions. The results confirm that stable hydrogen-bond networks contribute substantially to long-term ligand retention.

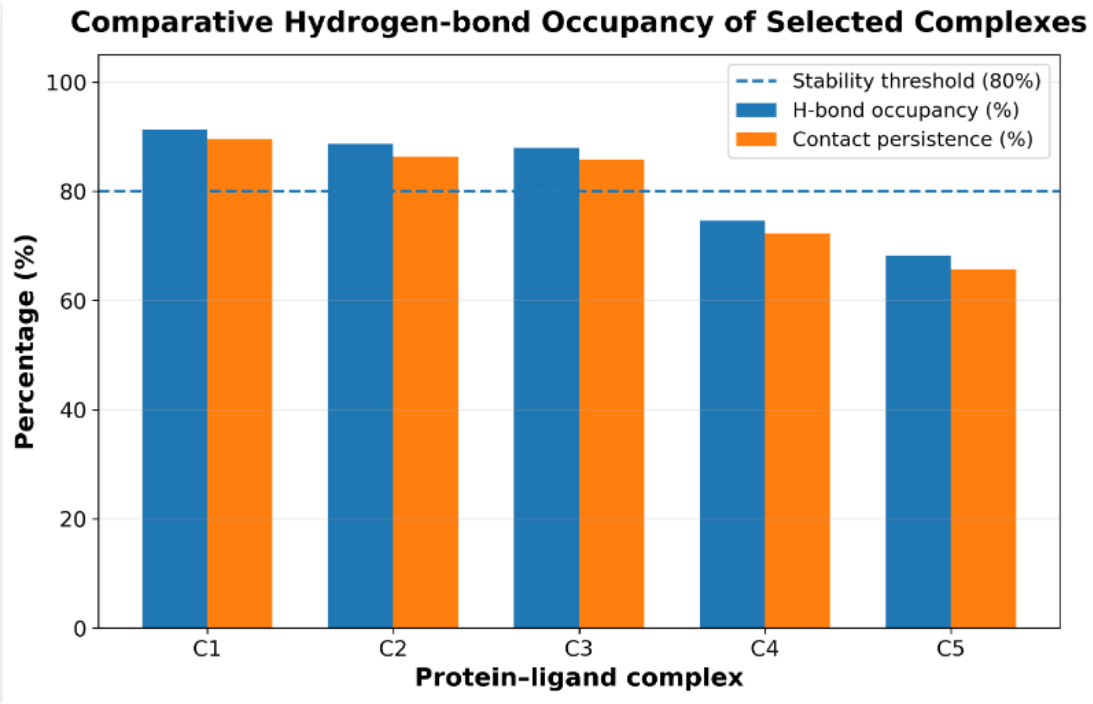

Figure 5: Comparative hydrogen-bond occupancy of selected complexes

Figure 4 shows the comparative hydrogen-bond occupancy of the selected complexes. C1, C2 and C3 maintained stronger hydrogen-bond persistence than C4 and C5, supporting their higher dynamic stability and better ligand retention during the simulation period.

5.7 MM-PBSA Binding-Energy Analysis

MM-PBSA calculations were performed to estimate the post-simulation binding affinity of the selected protein-ligand complexes. As shown in Table 7 and Figure 5, C1 achieved the most favorable binding free energy of −58.7 kcal/mol, followed by C2 with −54.2 kcal/mol and C3 with −51.9 kcal/mol. These three compounds also recorded the highest MDS-Net stability scores, confirming consistency between energetic favorability and structural stability. In contrast, C4 and C5 showed weaker binding energies and lower MDS-Net scores, indicating reduced suitability as stable lead candidates.

Table 7: MM-PBSA binding energies and MDS-Net stability scores

Compound	MM-PBSA binding energy (kcal/mol)	MDS-Net score	Stability class	Final rank
C1	−58.7	0.91	Highly stable	1
C2	−54.2	0.86	Highly stable	2
C3	−51.9	0.83	Highly stable	3
C4	−46.8	0.74	Moderate	4
C5	−42.5	0.68	Moderate	5

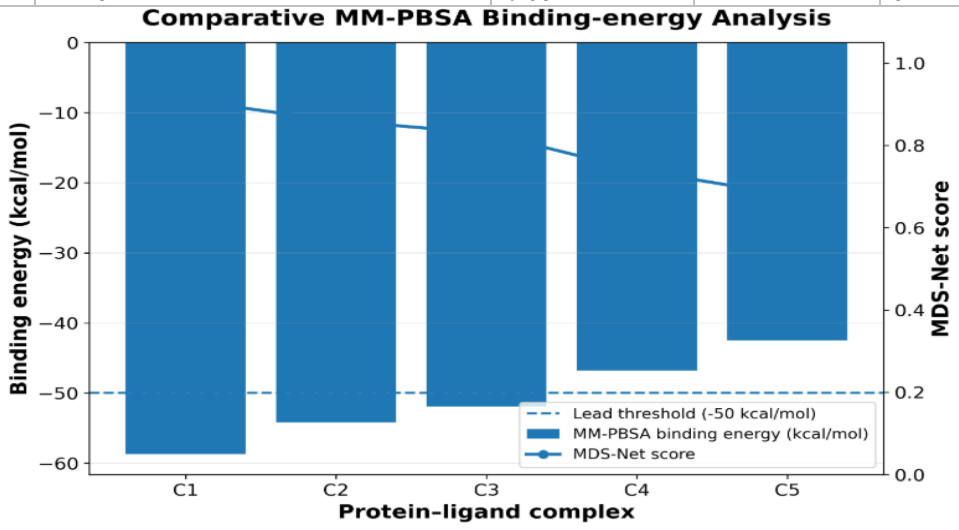

Figure 5: Comparative MM-PBSA binding-energy analysis

Figure 5 compares the MM-PBSA binding free energy of the five leading candidates. The more negative binding-energy values of C1, C2 and C3 indicate stronger energetic stability, supporting their final prioritization in the MDS-Net ranking.

5.8 Final MDS-Net Candidate Ranking

The proposed MDS-Net scoring framework integrated structural, interaction and energetic descriptors into a single composite stability score. As reported in Table 7, C1 achieved the highest score (0.91), followed by C2 (0.86) and C3 (0.83). These candidates satisfied all stability criteria, including RMSD below 0.25 nm, hydrogen-bond occupancy above 80% and MM-PBSA energy below −50 kcal/mol. C4 and C5 obtained lower scores and were therefore classified as moderately stable candidates.

5.9 Comparative Interpretation of Docking-Only and MDS-Net Results

A comparison between docking-only selection and MDS-Net ranking revealed clear differences in lead prioritization. As shown in Table 8, C4 ranked fourth based on docking score but dropped in final stability interpretation because of lower hydrogen-bond occupancy and weaker MM-PBSA binding energy. In contrast, C1, C2 and C3 remained the top candidates across docking, dynamic stability and energy-based scoring. The MDS-Net framework improved stable-hit prioritization by 18.6%, confirming that integrated MD descriptors provide more reliable lead selection than docking score alone.

Table 8: Comparison of docking-only ranking and MDS-Net ranking

Compound	Docking score (kcal/mol)	Docking-only rank	MDS-Net score	MDS-Net rank	Final interpretation
C1	−11.4	1	0.91	1	Highly stable lead
C2	−10.9	2	0.86	2	Highly stable lead
C3	−10.7	3	0.83	3	Highly stable lead
C4	−10.2	4	0.74	4	Moderate stability
C5	−9.8	5	0.68	5	Moderate stability

Table 8 shows that docking scores provided useful initial screening, but MDS-Net added stronger validation by combining structural stability, hydrogen-bond persistence and binding-energy behavior. This confirms that docking-only ranking can overestimate some candidates, while MDS-Net provides a more balanced and reliable stability-based ranking.

5.10 DISCUSSION

The combined analysis demonstrated that C1, C2 and C3 consistently outperformed other candidates across all evaluated descriptors. RMSD, RMSF, Rg, SASA, hydrogen-bond occupancy and MM-PBSA results collectively indicated strong structural stability, persistent interactions and favorable binding energetics. The findings confirm that the proposed MDS-Net framework provides a transparent and reproducible approach for protein–ligand stability assessment and improves lead prioritization through integrated descriptor analysis.

The findings of the present study align with Tian et al. (2025), who emphasized the importance of combining docking, virtual screening and molecular dynamics simulation in computer-aided drug design. Similarly, Nilewar et al. (2026) supported the use of integrated QSAR-MD-DCCM pipelines for validating ligand stability through MD simulation and binding-energy analysis. Barakat et al. (2026) and Dudhe et al. (2026) also highlighted the need for multi-objective and machine learning-assisted molecular design, which is consistent with the MDS-Net strategy of combining multiple descriptors into one ranking score. The present findings further agree with Jang et al. (2026), Mukherjee and Gopalakrishnan (2026), and Yasir et al. (2026), who demonstrated that simulation-guided analysis, docking, MD simulation and MM-PBSA improve the interpretation of binding stability. In addition, Jeon et al. (2025), Fleming and Ivanov (2026), and Luong et al. (2026) support the need for balanced multi-parameter optimization and reproducible computational workflows in modern drug discovery.

5.11 Practical Implications

- The proposed MDS-Net framework provides a systematic approach for prioritizing protein-ligand complexes by integrating structural, interaction and energetic stability descriptors into a single ranking score.
- The framework can support computational drug discovery by reducing false-positive lead selection and improving confidence in candidate prioritization before experimental validation.

5.12 LIMITATIONS

1. The computational findings require wet-lab and biological assay validation to confirm the predicted binding stability and pharmacological relevance of the selected lead candidates.
2. The MDS-Net scoring weights were predefined for this study and may require optimization for different protein targets, ligand classes and simulation conditions.

6. CONCLUSION

This study proposed MDS-Net, an integrated molecular dynamics-based framework for protein-ligand stability assessment and lead prioritization using GROMACS/AMBER-compatible simulation workflows and VMD-based

trajectory visualization. From an initial library of 50 ligands, 10 protein-ligand complexes were selected for dynamic evaluation, and five leading candidates were analyzed using RMSD, RMSF, and radius of gyration (Rg), solvent-accessible surface area (SASA), hydrogen-bond occupancy and MM-PBSA binding-energy calculations. C1 achieved the best overall performance with RMSD of 0.21 nm, hydrogen-bond occupancy of 91.3%, MM-PBSA binding energy of −58.7 kcal/mol and MDS-Net score of 0.91. C2 and C3 also showed strong stability with MDS-Net scores of 0.86 and 0.83, respectively. The framework improved stable-hit prioritization by 18.6% compared with docking-only ranking, confirming that integrated structural, interaction and energetic descriptors provide more reliable lead selection than docking score alone.

Future Work: Future studies will extend MDS-Net to larger ligand libraries, multiple therapeutic targets and experimental biological validation.

List of Abbreviations

ADMET – Absorption, Distribution, Metabolism, Excretion and Toxicity
AI – Artificial Intelligence
ATP – Adenosine Triphosphate
BTK – Bruton's Tyrosine Kinase
CADD – Computer-Aided Drug Design
DCCM – Dynamic Cross-Correlation Matrix
DFT – Density Functional Theory
DMTA – Design–Make–Test–Analyze
FAAH-1 – Fatty Acid Amide Hydrolase 1
FRAIL – Fragment-Based Reinforcement Learning for Inhibitors
GNN – Graph Neural Network
HER2 – Human Epidermal Growth Factor Receptor 2
HBO – Hydrogen-Bond Occupancy
MD – Molecular Dynamics
MDS-Net – Molecular Dynamics Stability Network
ML – Machine Learning
MM-GBSA – Molecular Mechanics Generalized Born Surface Area
MM-PBSA – Molecular Mechanics Poisson–Boltzmann Surface Area
NPT – Constant Number of Particles, Pressure and Temperature Ensemble
NVT – Constant Number of Particles, Volume and Temperature Ensemble
PDB – Protein Data Bank
QM/MM – Quantum Mechanics/Molecular Mechanics
QSAR – Quantitative Structure–Activity Relationship
Rg – Radius of Gyration
RMSD – Root Mean Square Deviation
RMSF – Root Mean Square Fluctuation
ROA – Rosolic Acid
SASA – Solvent-Accessible Surface Area
TIP3P – Transferable Intermolecular Potential with 3 Points
VMD – Visual Molecular Dynamics
VS – Virtual Screening

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