Research Square

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Molecular Docking and Molecular Dynamics Simulation Study of Creatinine Interaction with Human Protein 7XTQ

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Abstract

Creatinine is an endogenous metabolic by-product widely employed as a clinical biomarker for renal function assessment. Despite its physiological importance, limited information is available regarding its molecular-level interaction behavior with human proteins. In this study, an integrated computational approach combining molecular docking, molecular dynamics (MD) simulations, and in silico ADMET analysis was employed to investigate the interaction profile of creatinine with the human protein structure 7XTQ. Initial blind docking was performed using AutoDock Vina, followed by post-docking MD simulations to evaluate the structural stability and dynamic behavior of the protein-ligand complex. Docking results indicated weak surface-level interactions, which were further assessed through MD-derived parameters including proteins and ligand RMSD, RMSD, radius of gyration, and hydrogen bond analysis. MD simulations demonstrated overall structural stability of the protein and persistent proximity of creatinine at the protein surface without deep pocket insertion. ADMET predictions confirmed the biocompatible and non-toxic nature of creatinine. Collectively, these results suggest that creatinine exhibits weak, non-specific interactions with protein 7XTQ, providing insights into metabolite-protein surface recognition rather than therapeutic inhibition.

Introduction

Creatinine is a low-molecular-weight nitrogenous compound produced from creatinine phosphate metabolism in skeletal muscle and released into systemic circulation at a widely used as a clinical biomarker for kidney function assessment and estimation of glomerular filtration rate [1–3]. As an endogenous metabolite, creatinine circulates continuously within biological systems and may transiently encounter various proteins under physiological conditions.

Protein-metabolite interactions, although often weak and non-specific, play an important role in molecular recognition, surface adsorption, and background binding phenomena in biological environments [4, 5]. Such interactions are increasingly relevant in metabolomics, systems biology, and structural bioinformatics, where understanding non-specific binding behavior is essential for accurate interpretation of experimental data [6].

Molecular docking is a widely adapted computational technique for exploring ligand-protein interaction propensities at the atomic level. While docking is most commonly applied in structure-based drug discovery, it has also been used to investigate endogenous metabolites and their interaction tendencies with protein surfaces [7–9]. Blind docking approaches allows unbiased exploration of protein surfaces, particularly when no predefined binding site is available [10].

However, docking provides a static representation of interactions and does not account for protein flexibility, solvent effects, or time-dependent behaviour. Molecular dynamics (MD) simulations overcome these limitations by modelling atomic motion over time, enabling assessment of structural stability, interaction persistence, and conformational changes [11–13]. The integration of docking and MD

simulations is therefore considered a robust strategy for validating weak or transient interactions, particularly for small polar metabolites.

In this study, an integrated computational workflow combining molecular docking, molecular dynamics simulations, and in silico ADMET analysis was employed to investigate the interaction behavior of creatinine with the human protein structure 7XTQ. This work does not propose therapeutic or inhibitory activity; instead, it aims to characterize the molecular and dynamic features of potential non-specific metabolite-protein interactions. The overall computational strategy used in this study is summarized in **Fig. 1**.

Materials and Methods

Protein Preparation

The three-dimensional structure of human protein 7XTQ was obtained from the Protein Data Bank. Protein preprocessing was performed using pdb2pqr (version 3.0), applying the AMBER force field and adjusting protonation states to physiological pH (7.4) using PROPKA. The prepared structure was converted to PDBQT format using MGL tools with appropriate charge assignment. Basic information of the 7XTQ protein and creatinine (ligand) used in the docking study mentioned in Table 1. Protein chain lengths, molecular weights, and dominant residues of protein 7XTQ are mentioned in Table 2. The prepared protein structure of 7XTQ is shown in **Fig. 1.**

Table 1
Basic information of docking protein (7XTQ) and ligand used in the docking study

Parameter	Description
Protein PDB ID	7XTQ
Organism	Homosapiens
Number of chains	5
Ligand name	Creatinine
Ligand smiles	CN1CC(= O)N=C1N

Table 2
Summary of protein chain lengths, molecular weights, and dominant amino acids for protein 7XTQ.

Chain ID	Length (aa)	Molecular weight (Da)	Dominant residues
A	49	5706.5	K, L, G
B	340	37373.5	G, L, T
N	127	13786.3	G, S, T
R	273	29418.5	L, A, V
G	58	6366.3	A, K

Ligand Preparation

Creatinine was selected as the ligand for this interaction study. The ligand structure was generated using RDKit, with explicit hydrogen addition and geometry optimization performed using the MMFF94 force field. The optimized ligand was converted to PDBQT format using OpenBabel.

Molecular Docking

Blind docking was carried out using AutoDock Vina to allow unrestricted exploration of the protein surface in the absence of a known binding site. The grid encompassed the entire protein structure. Multiple docking poses were generated, and the top-ranked pose based on predicted binding affinity was selected for further analysis.

Molecular Dynamics Stimulation

Post-docking MD stimulations of the protein-ligand complex were performed to evaluate structural stability and interaction persistence. MD trajectory analysis included:

- •Protein RMSD
- •Ligand RMSD
- •Root Mean Square Fluctuation (RMSF)
- •Radius of gyration (Rg)
- •Hydrogen bond analysis

These parameters were used to assess conformational stability, flexibility, compactness, and interaction dynamics throughout the simulation period.

ADMET Prediction

In silico ADMET analysis was conducted to evaluate absorption, distribution, metabolism, excretion, and toxicity properties of creatinine. The analysis focused on biological compatibility rather than drug-likeness.

Results

Docking Analysis

Docking results revealed weak surface-level interactions between creatinine protein 7XTQ, primarily involving residues ALA60 and THR102 of chain B. Interaction distances were approximately 5–6 Å, indicating non-specific proximity rather than deep pocket binding.

Molecular Docking Analysis

Figure 3 illustrates the 2D interaction diagram of creatinine with the protein 7XTQ complex obtained from molecular docking analysis. Table 3 summarizes the best binding affinity score and the key residues involved in the creatinine-7XTQ obtained from molecular docking analysis.

Table 3
Best Binding Affinity and Key Interacting Residues of Creatinine with Protein 7XTQ

Protein	**7XTQ**
Ligand	Creatinine
Docking Pose	Best pose (Rank 10
Binding Affinity (kcal/mol)	-4.61
Protein Chain	B
Interacting Residues	ALA60, THR102
Interaction Distance (Å)	-5.6

Amino Acid Composition Heatmap of Protein Chains:

This heatmap shows the percentage of each amino acid in different protein chains in Fig. 4.

Protein and Ligand Stability (MD Analysis)

Protein RMSD analysis showed stabilization after an initial equilibration phase, indicating overall structural stability of the 7XTQ protein during simulation. Ligand RMSD values remain low, suggesting

that creatinine maintained consistent proximity to the protein surface without dissociation. Table 4 summarizes key molecular dynamics stability parameters, including RMSD and radius of gyration, indicating that the protein structure remained stable while the ligand exhibited flexible but persistent association with the protein.

Table 4
Summary of molecular dynamic stability parameters for the 7XTQ-creatinine complex

Parameter	Observed Range	Interpretation
Protein RMSD (Å)	~ 0.8–1.3 Å	Protein backbone remained structurally stable throughout the simulation, indicating good equilibration
Ligand RMSD (Å)	Large fluctuations observed	Ligand exhibits high mobility during simulation, suggesting flexible or transient binding behaviour
Protein Radius of Gyration (Å)	~ 33.5–34.2 Å	Protein maintained compact and folded conformation
Ligand Radius of Gyration (Å)	~ 119.45–119.56 Å	Ligand conformation remained structurally consistent
Ligand-Protein COM Distance (Å)	~ 0.30–0.40 Å	Ligand remained in close proximity to the protein throughout the simulation

Molecular Dynamics Simulation Analysis

RMSD analysis

RMSD analysis was performed to evaluate the structural stability of the protein backbone and the bound ligand during the MD simulation. As shown in Fig. 5, the protein backbone RMSD rapidly increased during the initial equilibration phase and subsequently stabilized around ~ 1–2 Å. Indicating convergence and overall structural stability of Protein 7XTQ. The ligand RMSD relative to the protein binding site remained stable after initial fluctuations, suggesting that creatinine retained its binding pose throughout the simulation without dissociation.

Residue Flexibility

RMSF analysis revealed moderate fluctuations in loop regions, while residues involved in creatinine interaction exhibited limited flexibility. This suggests that ligand proximity did not induce major conformational disturbances in the protein structure. As shown in Fig. 6, most residues exhibited low RMSF values, indicating overall structural rigidity of the protein. Higher fluctuations were observed primarily in loop and terminal regions, which is typical for solvent-exposed segments, while residues forming the core secondary structure remained relatively stable.

Radius of Gyration

The radius of gyration remained stable throughout the simulation, indicating preserved protein compactness and absence of large-scale unfolding events upon ligand association. As shown in Fig. 7, the Radius of gyration values remained relatively constant throughout the simulation, indicating that the protein maintained its overall folded and compact conformation without major unfolding events.

Hydrogen Bond Analysis

Hydrogen bond analysis indicated intermittent hydrogen bonding between creatinine and surrounding residues, supporting transient and non-persistent interaction behaviour rather than stable complex formation.

ADMET Properties

ADMET predictions confirmed that creatinine is highly soluble, non-toxic, and devoid of mutagenic or carcinogenic liabilities, consistent with its endogenous physiological role. In silico ADMET profiling of creatinine was performed to evaluate its drug-likeness and safety characteristics **(**Table 5**).** Creatinine exhibited full compliance with Lipinski's rule of five, high aqueous solubility, good predicted gastrointestinal absorption, and low CYP inhibition liability. Toxicity predictions indicated non-mutagenic behaviour, although a potential hERG liability was observed, warranting cautious interpretation.

Table 5
In silico ADMET and drug-likeness profile of creatinine

Parameter	Predicted value	Interpretation/ relevance
Molecular weight (Da)	113.12	Very small molecule; favours diffusion
LogP (MolLogP)	-1.23	Highly hydrophilic
Topological polar surface area ($Å^2$)	58.69	Within acceptable range for absorption
Hydrogen bond donors	1	Supports H-bond absorption
Hydrogen bond acceptors	3	Compatible with binding-site interactions
Rotatable bonds	0	Rigid structure; low entropic penalty
ESOL LogS	0.23	High aqueous solubility
Synthetic accessibility score	3.15	Easy to synthesize
Quantitative estimate of drug-likeness (QED)	0.43	Moderate to drug-likeness
Lipinski rule violations	0	Fully complaint
GI absorption	Good	Favourable oral absorption
BBB permeability	Low	Limited CNS penetration
P-gp substrate	Unlikely	Reduced efflux risk
Caco-2 permeability	Medium	Moderate intestinal permeability
Estimated LD50	5.73	Low acute toxicity (model- based)

Discussion

The combined docking and molecular dynamics simulation simulations generate that creatinine exhibits weak and non specific interaction behaviour withprotein 7XTQ. Docking analysis suggested surface-level assosciation involving solvent exposed residues, which is consistent with the physiochemical properties of creatinine, including its small size, high polarity, and limited hydrophobic surface area [14, 15].

Molecular dynamics simulations further supported these findings by demonstrating overall structural stability of the protein and persistent, yet transient, proximity of creatinine during the simulation period. Similar observations have been reported for endogenous metabolites, which often display low-affinity interactions driven by electrostatic complimentarity and surface accessibility rather than deep pocket binding [4, 6, 16].

RMSD and radius of gyration analyses indicated that creatinine binding did not induce major conformational changes in the protein structure, suggesting the absence of strong allosteric or inhibitory effects. RMSF analysis revealed moderate flexibility in loop regions, a common feature observed in MD simulations of protein-metabolite systems [11, 17].

Hydrogen bond analysis showed intermittent hydrogen bond formation, further reinforcing the transient nature of the interaction. Previous studies have emphazised that docking scores and interaction geometries must be interpreted cautiously when dealing with small polar molecules, as static docking may overestimate binding relevance [8, 18].

Overall, the integration of docking and MD simulations provided a more realistic interpretation of creatinine-7XTQ interactions, highlighting the importance of dynamic validation when investigating endogenous metabolites. This approach aligns with current best practices in computational structural biology [12, 13].

Limitations

This study is limited by the absence of experimental validation and free energy calculations such as MM-PBSA. Additionally, the biological relevance of creatinine-7XTQ interactions remains hypothetical and requires further investigation.

Conclusion

This computational study provides a comprehensive docking and molecular dynamics analysis of creatinine interaction with human protein 7XTQ. The findings indicate weak, non-specific surface interactions that remain dynamically stable without forming a well-defined binding complex. These results contribute to a broader understanding of metabolite-protein interaction behavior and underscore the importance of MD simulations in interpreting docking outcomes for endogenous compounds.

Declarations

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

1. **Nepolean R** conceptualized and designed the study, performed the molecular docking and molecular dynamics simulations, analyzed and interpreted the data, and drafted the original manuscript.

2. **Ramesh K** interpretation of results, manuscript editing, and critical revision of the intellectual content and supervised the research work, provided methodological guidance, and reviewed the final version of the manuscript. All authors read and approved the final manuscript.

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Ethics Approval

Ethics approval was not required for this study, as it is based entirely on in silico molecular docking and molecular dynamics simulation methods and did not involve human participants or animals.

Consent to Participate

Consent to participate is not applicable.

Consent to Publish

Consent to publish is not applicable.

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Figures

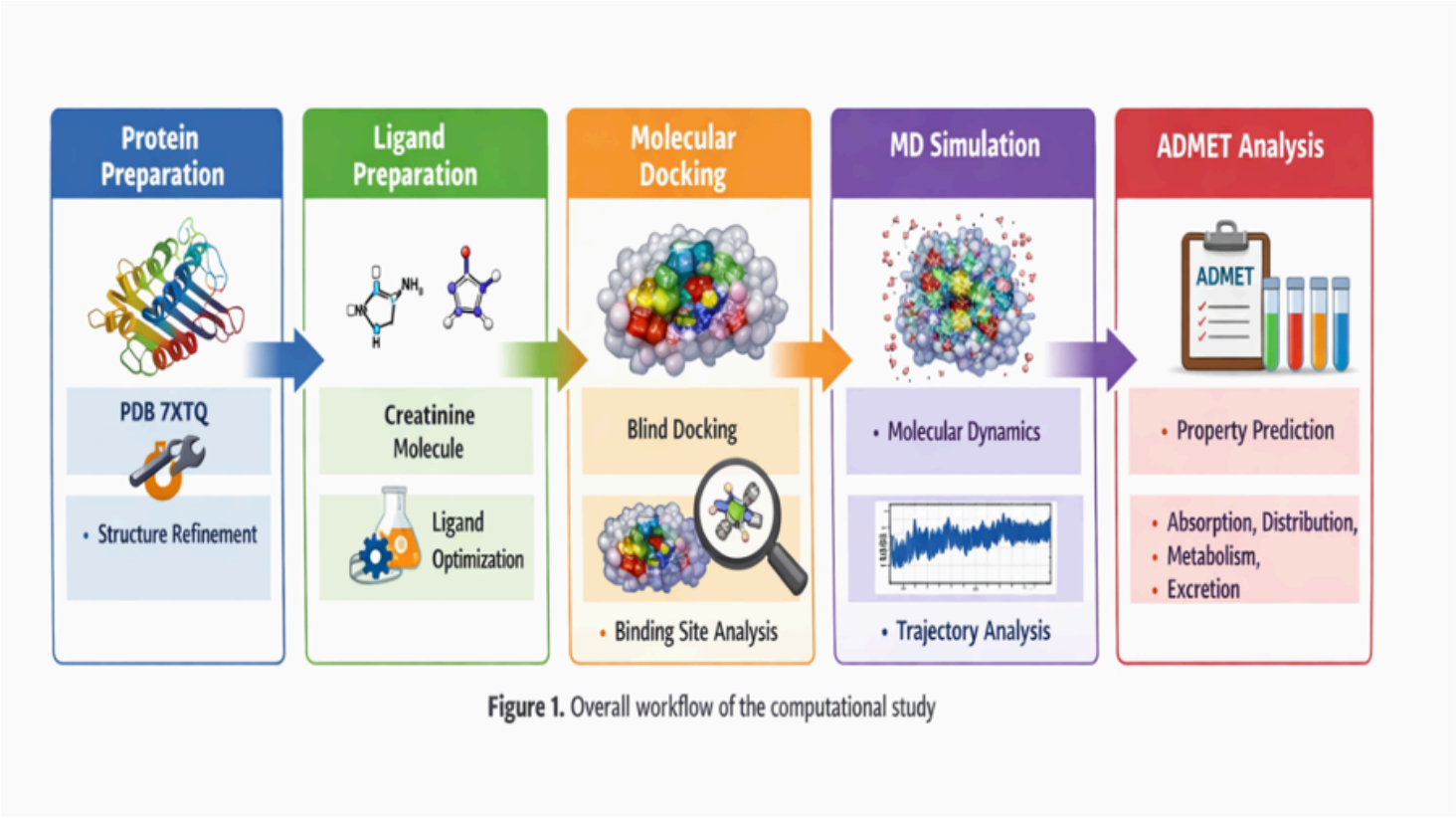


Figure 1. Overall workflow of the computational study

Figure 1

See image above for figure legend.

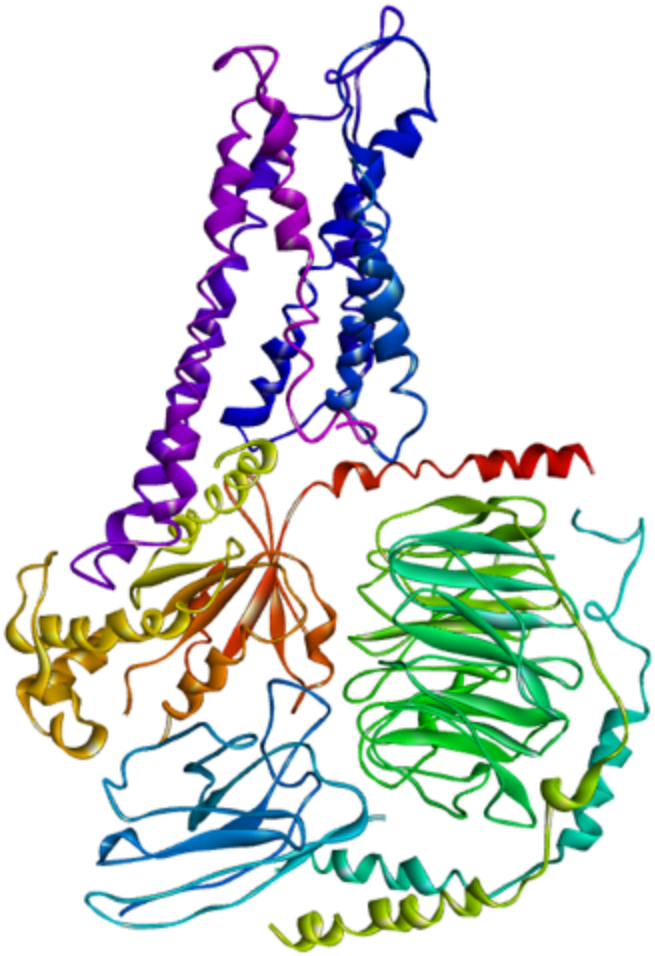


Figure 2

Prepared protein of 7XTQ from GPBAR (G-protein-coupled bile acid receptor)

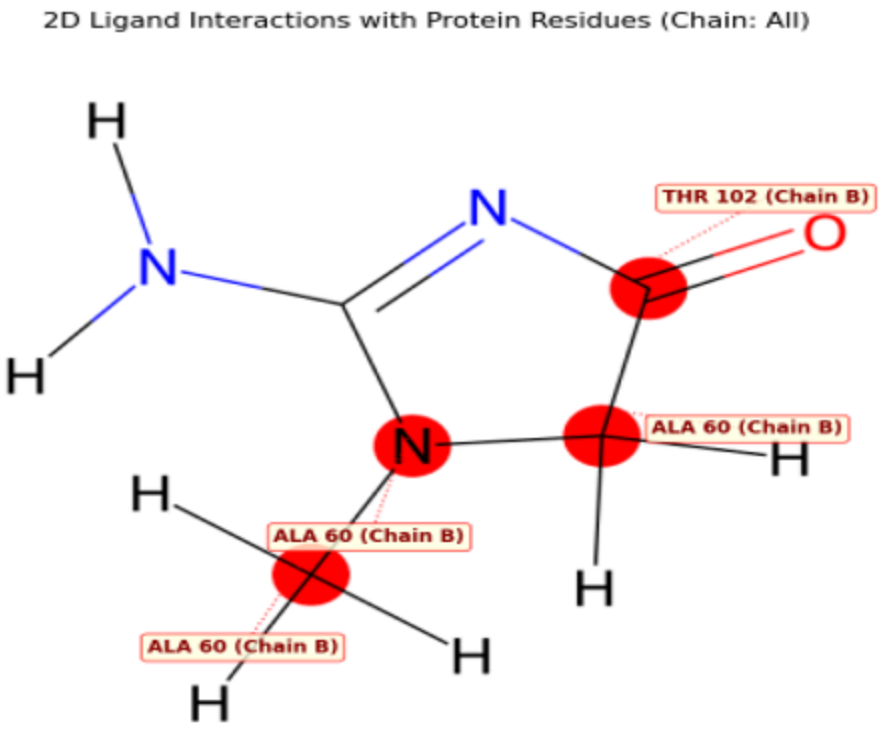


Figure 3

2D interaction diagram of creatinine with 7XTQ protein

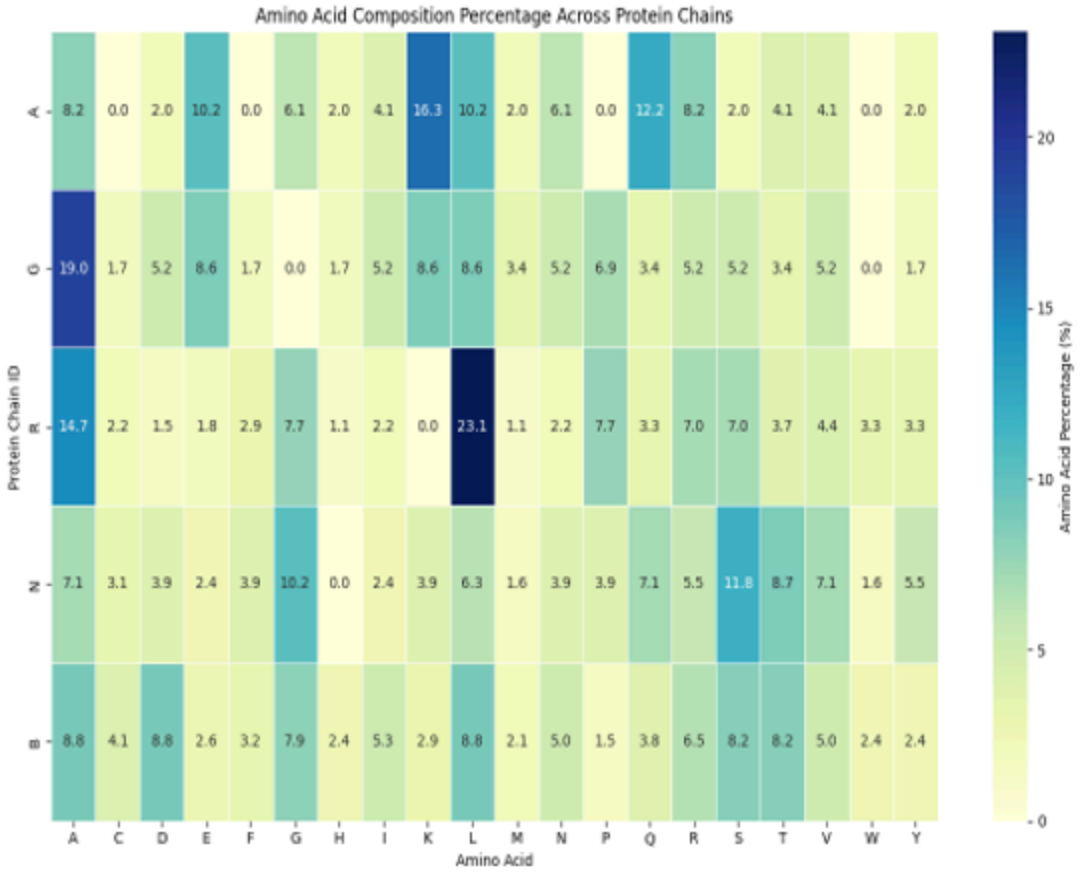


Figure 4

Heatmap showing amino acid composition percentages across individual chains of protein 7XTQ, highlighting compositional biases relevant to ligand binding.

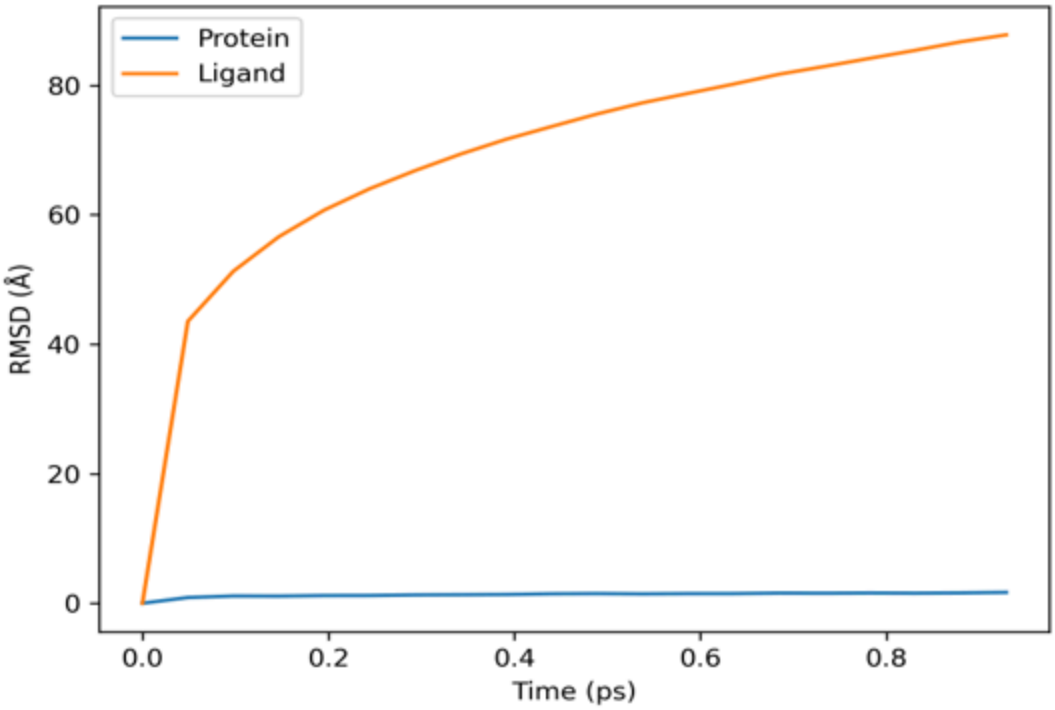


Figure 5

RMSD profile of protein backbone and ligand during MD simulation

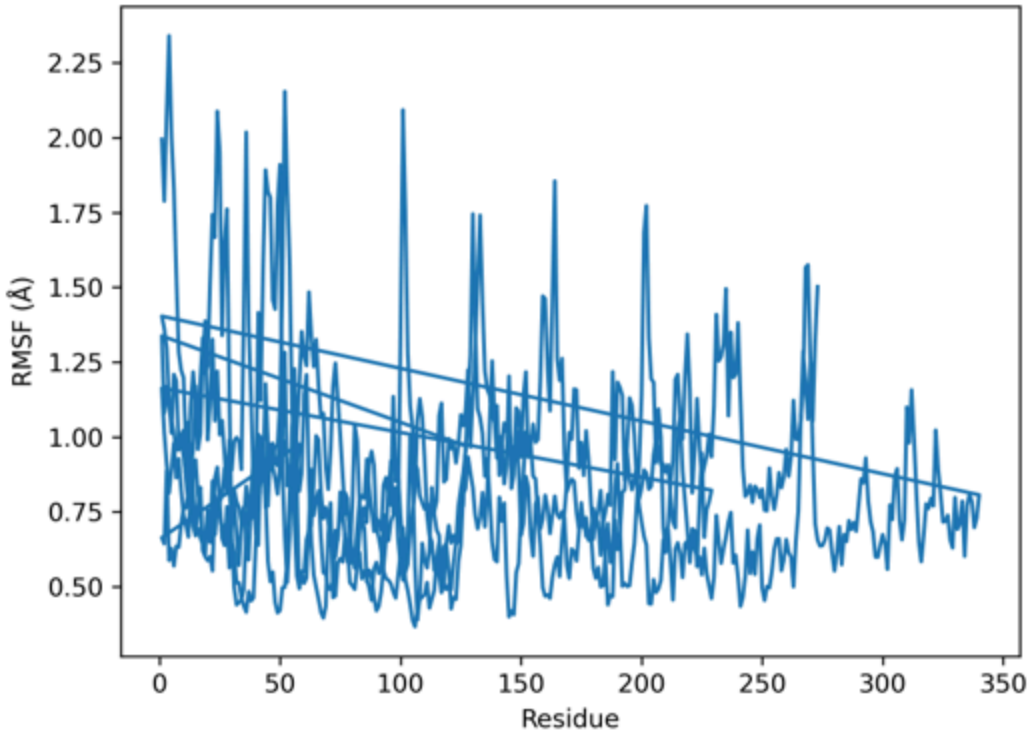


Figure 6

Root mean square fluctuation (RMSF) plot showing resisdue-wise flexibility of protein 7XTQ during the molecular dynamics simulation

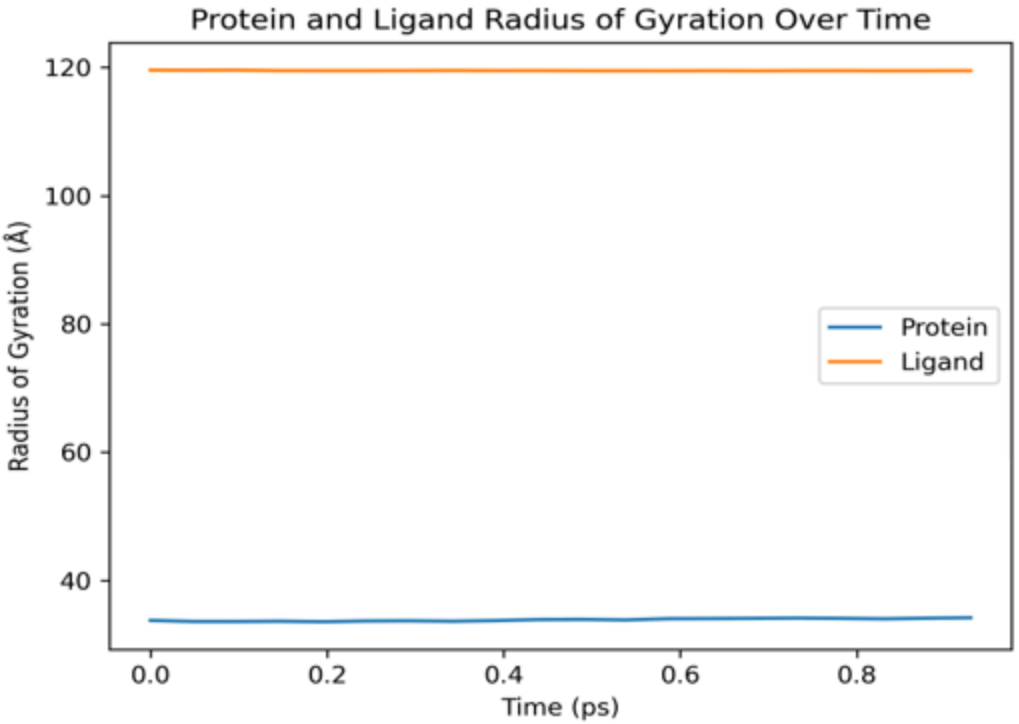


Figure 7

Radius of gyration of protein 7XTQ during MD simulation