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# Molecular Dynamics Simulations In Docking Metal Binding Site And Free Energy Calculation

**Pardhasarathi M[1] and J. Naveena Lavanya Latha***

**[1]KL University, Guntur**

**[2*]Department of Biosciences and Biotechnology, Krishna University, Machilipatnam**

## Abstract

This study investigates the dynamic behavior and stability of proteins and protein-ligand complexes through molecular dynamics (MD) simulations using the Desmond Molecular Dynamics system and the OPLS 2005 all-atom force field. The simulations aimed to explore the conformational changes, fluctuations, and overall stability of proteins in both their unbound forms and when complexed with ligands over a 10-nanosecond period. To assess the binding affinity and energetics of the protein-ligand complexes, the Prime/MM-GBSA method was employed, which calculates the binding free energies (ΔG binding) by considering various energy components, including the energy of the complex, solvation energy difference, and surface area energy difference. The study utilized the OPLS-AA force field and the GB/SA continuum solvent model to estimate the binding free energies, providing insights into the stability and strength of the receptor-ligand interactions, which are crucial for identifying potential drug candidates and optimizing ligands for enhanced binding affinity.

## Introduction

Molecular dynamics (MD) simulations have become an indispensable tool in the field of computational chemistry and structural biology, allowing researchers to explore the dynamic behavior of biological macromolecules at an atomic level. By simulating the physical movements of atoms and molecules over time, MD simulations provide detailed insights into the conformational changes, stability, and interactions of proteins, nucleic acids, and other biomolecules. The method relies on solving Newton's equations of motion for a system of interacting particles, where the forces between atoms are derived from a pre-defined force field such as the Optimized Potentials for Liquid Simulations (OPLS) all-atom force field (Jorgensen et al., 1996). MD simulations are particularly valuable for studying protein-ligand interactions, as they allow for the examination of how a ligand binds to a protein and how this binding affects the protein's structure and function over time (Karplus & Kuriyan, 2005).

One of the key challenges in drug discovery is accurately predicting the binding affinity of potential drug candidates to their target proteins. The Prime/MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) method is a widely used approach for estimating the binding free energy of protein-ligand complexes (Gohlke & Case, 2004). This method combines molecular mechanics energies with solvation effects modeled by the Generalized Born (GB) and Surface Area (SA) terms. Prime/MM-GBSA calculations are often performed following molecular docking studies and MD simulations to refine binding poses and predict the strength of protein-ligand interactions (Lyne, 2002). The accuracy and efficiency of Prime/MM-GBSA make it a popular choice for virtual screening and lead optimization in drug discovery programs.

In MD simulations, the dynamic properties of proteins and their complexes with ligands are explored by simulating their behavior over a specific time period, typically ranging from nanoseconds to microseconds. The resulting trajectories provide information on how the protein and ligand conformations evolve, revealing critical aspects of molecular recognition, binding stability, and potential drug efficacy (Lindahl et al., 2020). By coupling MD simulations with Prime/MM-GBSA, researchers can obtain a comprehensive view of both the structural dynamics and thermodynamics of protein-ligand interactions, thus enabling more informed decisions in the drug design process.

**Molecular dynamics simulations**

To investigate the dynamic behavior and stability of the proteins and protein-ligand complexes, molecular dynamics (MD) simulations were performed using the Desmond Molecular Dynamics system. The simulations utilized the Optimized Potentials for Liquid Simulations (OPLS) all-atom force field, specifically the 2005 version.

The prepared structures were then input into the Desmond set-up wizard to configure the necessary parameters for the MD simulations.

The MD simulations were carried out to explore the dynamic behavior of the proteins in their unbound forms, as well as to investigate the stability of the docking of ligands into the modeled protein structures. By subjecting the protein-ligand complexes to MD simulations, the interactions between the protein and ligand were examined over a period of 10 nanoseconds (ns). Similar simulation parameters, as described previously, were employed to ensure consistency across the experiments.

During the MD simulations, the system was subjected to a series of computational steps, where the forces and energies governing the atomic interactions were calculated and updated at each time step. This allowed for the exploration of the conformational changes, fluctuations, and overall stability of the protein structures and protein-ligand complexes over the simulation time.

**Prime/MM-GBSA binding-free energy calculation**

To assess the binding affinity and energetics of the protein-ligand complexes, the Prime/MM-GBSA method was employed. This method allows for the calculation of binding free energies by utilizing the output post-viewer files obtained from the XP docking protocol. These files serve as input for the Prime/MM-GBSA protocol, which is used to estimate the binding free energy (ΔG binding) of the complexes. The binding free energy (ΔG binding) is determined based on several energy components, including the energy of the complex (ER:L), the sum of energies of the receptor and ligand in their unbound states (ER + EL), the solvation energy difference (ΔGsolv), and the surface area energy difference (ΔGSA).

The energy of the complex (ER:L) represents the total energy of the protein-ligand complex, taking into account their interactions and structural arrangement. The sum of energies of the receptor and ligand in their unbound states (ER + EL) accounts for the individual energies of the unbound components. The solvation energy difference (ΔGsolv) considers the variation in the GBSA (Generalized Born Surface Area) solvation energy between the complex and the sum total of solvation energies of the unbound receptor and ligand. This component reflects the changes in the solvent interactions upon complex formation.

The surface area energy difference (ΔGSA) accounts for the difference in surface area energies between the complex and the sum total of surface area energies of the unbound receptor and ligand. This factor considers the changes in the exposed surface areas of the molecules upon complex formation. To calculate these energies, the OPLS-AA (Optimized Potentials for Liquid Simulations, all-atom) force field and the GB/SA (Generalized Born/Surface Area) continuum solvent model were utilized. The OPLS-AA force field accounts for the atomic interactions and force field parameters, while the GB/SA solvent model takes into consideration the solvation effects in a continuum solvent approximation.

By applying the Prime/MM-GBSA method, the binding free energies of the protein-ligand complexes were estimated, providing valuable insights into the stability and strength of the interactions between the receptor and ligand. These calculations aid in the identification of potential drug candidates and guide the optimization of ligands for enhanced binding affinity.

**Results**

**Virtual docking of putative cell wall proteins with calcium and strontium ions**

The prepared structures of calcium and strontium ions were subjected to docking simulations with the stabilized structures of cell wall proteins NCU07523 (CWP-A) and NCU08936 (CWP-B). The docking results revealed that both calcium and strontium ions exhibited nearly equal binding affinities with both CWPs. Specifically, calcium and strontium ions bound to NCU07523 (CWP-A) with Glide XP scores of -11.01 and -17.41, respectively. For NCU08936 (CWP-B), the corresponding scores were -8.2 and -9.8. The docking simulations also visualized the binding of these ligands to their respective binding pockets within the proteins.

It is important to note that while scoring functions used in docking programs provide valuable insights, they are not infallible. Therefore, additional validation of the docking results is necessary using other reliable approaches, such as free energy calculations and molecular dynamics (MD) simulations of the docked complexes. In this study, the PRIME free energy scores were calculated for CWP-A and CWP-B, resulting in values of 4518.257 and 3308.305 kcal/mol, respectively. The PRIME free energy of binding for the Ca-CWP-A complex was measured as 498.657 kcal/mol, while it was -81.791 kcal/mol for the Sr-CWP-A complex. Similarly, the PRIME free energy of binding for the Ca-CWP-B complex was 207.387 kcal/mol, and for the Sr-CWP-B complex, it was -221.464 kcal/mol.

From the docking scores, it was evident that strontium ions exhibited higher binding affinity with both CWPs compared to calcium ions. Although the difference between the docking scores and free energy values was minimal, it indicated that both calcium and strontium ions bound to NCU07523 and NCU08936 with almost equal affinity.

**Figure 1 Virtual docking of NCU07523 (CWP-A) with $Ca^{2+}$/$Sr^{2+}$ with binding pockets**

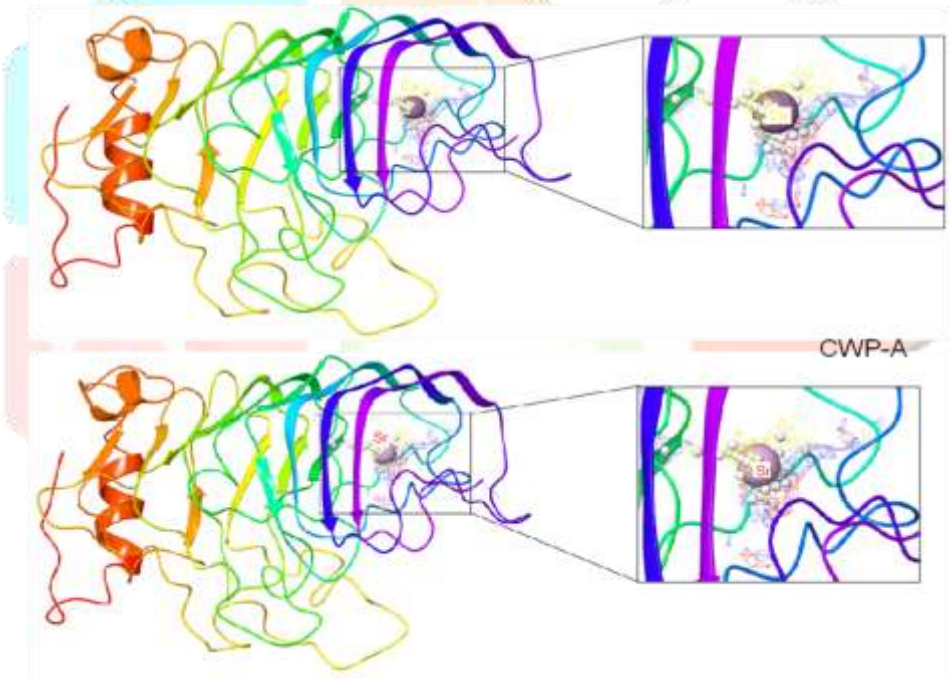
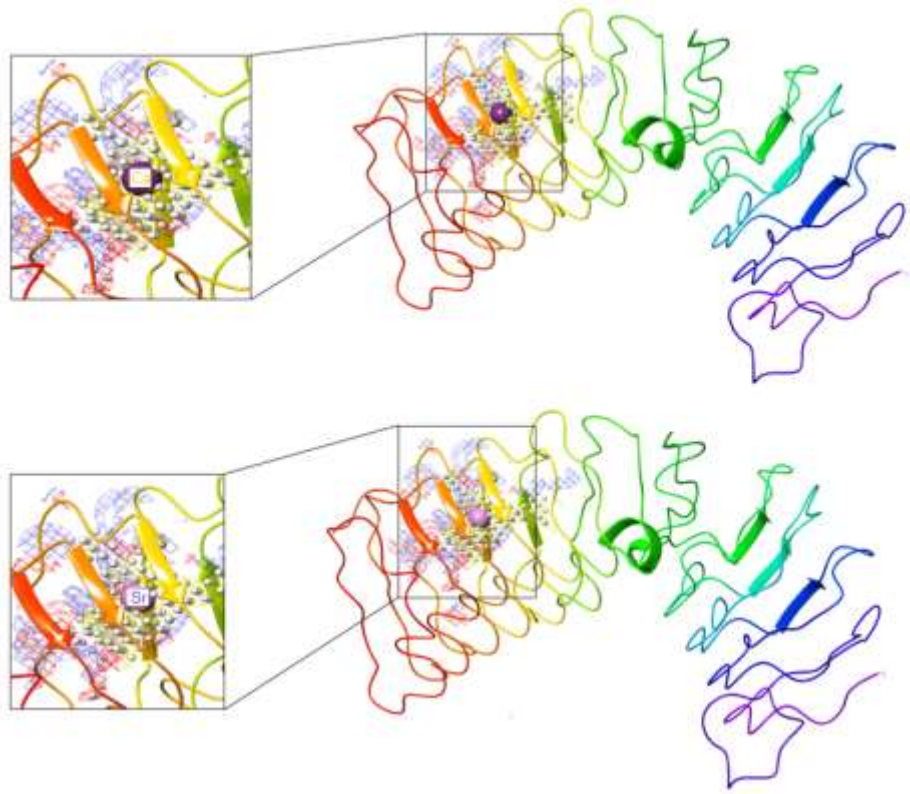


**Figure 2 Virtual docking of NCU08936 (CWP-B) with $Ca^{2+}$/$Sr^{2+}$ with binding pockets**



## Discussion

During molecular dynamics (MD) simulations, both calcium and strontium ions were found to be stable within the binding pockets of NCU07523 and NCU08936. Although lower PRIME free energies were observed for strontium compared to calcium during the MD simulation, both ions eventually acquired conformations that were no longer deviating from their initial positions by the end of the simulation. This suggests that both ions were able to maintain stable interactions within the binding pockets throughout the simulation period (Kennedy and Jones, 1994). The docking scores of calcium and strontium ions were found to be comparable after the docking process, indicating that both ions were able to interact with the predicted binding pockets of NCU07523 and NCU08936 accurately (Kota et al., 2022). These interactions involved hydrogen bonds and hydrophobic interactions with the residues present in the active site of the proteins. This further validates the accuracy of the predicted binding pockets and highlights the ability of both calcium and strontium ions to interact with the key residues within the protein's active site.

A comparison of the final binding free energies of NCU07523 and NCU08936 complexes with calcium and strontium ions revealed an interesting finding. The decrease in free energy of binding for strontium (a non-essential metal) was found to be the main reason for the stability of these proteins compared to that of calcium (an essential metal). This suggests that strontium, despite being a non-essential metal, is able to form stable interactions within the binding pockets of the proteins, potentially influencing their overall stability and function.

Overall, the integration of the implicit metal binding model with MD simulations and docking studies provided valuable insights into the stability and binding characteristics of calcium and strontium

ions within NCU07523 and NCU08936 proteins. These findings shed light on the differential effects of essential and non-essential metals on protein stability, highlighting the complex interplay between metal binding and protein function (Du et aal., 2010). Such information contributes to our understanding of metal-protein interactions and can aid in the design of metal-based therapeutics and the development of strategies for modulating protein function through metal binding.

Note: Unless specifically mentioned, all experiments were performed in duplicates and each experiment was conducted in triplicates. The data presented here are representative and display the average values obtained from the individual experiments. It is noteworthy that the variation between separate experiments was found to be less than 15%, indicating a high level of consistency and reliability in the results obtained.

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