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# Molecular Dynamics Simulation of *P.falciparum* Haemoglobinase Falcipain 2, in its apo and holo structural state

**T Rajguru[1*], D S Bora[2], M K Modi [3]**

[1]Research Scholar, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam
[2] Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam
[3] Professor, Department of Agricultural Biotechnology, AAU, Jorhat, Assam

**Abstract**

*Haemoglobinase in the food vacuole of the trophozoite stage of the malaria parasite Plasmodium falciparum are potent target for antimalarial drug discovery. In particular, falcipain 2(FP2), a cysteine protease, present in abundance in the food vacuole of the parasite is shown to be inhibited by a lead compound E64. We have performed Molecular Dynamics (MD) simulation studies of the apo structure and the referred holo structure of FP2 using Gromacs software. Structural and dynamic differences in the apo and holo structures were analyzed using pairwise distance distributions. The results were analyzed using the XmGrace package. These analyses reveal lesser backbone deviations in holo state (3 Å) than that in apo state (0.25-0.35) Å. Even the graphs of MD trajectories of holo state of FP2 showed decrease order of Radii of Gyration. Thus, it can be inferred that binding of the ligand leads to a more compact and stable conformations of the protein-ligand complex inducing more effective inhibition.*

**Key Words:** Molecular Dynamics, Falcipain, RMSD, P.falciparum, apo and holo enzyme.

## Introduction:

Despite important gains in some area, malaria remains to be one of the world's leading killer infectious diseases affecting around 200–300 million people and causing approximately 430,000 deaths every year globally [1].Widespread drug resistance increasingly limits the effectiveness of available therapies. New targets are required for the development of novel classes of antimalarial drugs [2].Trophozoite of *P. falciparum* hydrolyze hemoglobin in an acidic food vacuole to generate free amino acids essential for parasite survival [3, 4]. Among major haemoglobinase of *P. falciparum*, Falcipain-2 (FP-2) papain-family (C1A) cysteine

*Email: trisha.rajguru@gmail.com

proteases plays the major role in hemoglobin degradation and is produced earlier in the parasite lifecycle than the rest. This process of hemoglobin degradation is blocked by the cysteine protease inhibitors E64, as evidenced by the accumulation of undigested hemoglobin in the vacuole [5].

Since biological function of proteins is frequently associated with the formation of complexes with small-molecule ligands. Insight into these processes relies on detailed knowledge about the structure of protein/ligand complexes, e.g. how enzymes stabilize substrates and cofactors in close proximity. Moreover, almost all drugs are small-molecule ligands that interact with enzymes, receptors or channels. Accordingly, ligand-bound receptor complex structures are a critical prerequisite for understanding biological function and for structure based drug design [6].

Several studies showed that virtual screening to an apo-structure usually results in a poor enrichment factor (the ability to discriminate between binders and non-binders) compared to the holo-structure even when the structural difference between both is comparably small [7-9]. However, the degree of flexibility thus far is limited to either side chain motions or small variations of the backbone and thus, the availability of a holo-structure or an apo-structure that is highly similar to the holo conformation is currently a prerequisite for a successful docking, severely limiting structure-based drug design. Particularly, receptors that undergo a substantial conformational transition upon ligand binding are currently precluded from structure based drug design [10].

In the present work we have made a comparative study of the apo (without ligand) and the holo (with ligand) state of the enzyme falcipain 2 with its inhibitor E64 by thoroughly analyzing the derived MD trajectories.

## Materials and Methods:

The crystallographic structure file for apo and holo state of FP2 was retrieve from protein data bank (PDB ID 3BPF). To generate thermodynamically stable apo FP2, the E64 and other hetatms were removed from the E64 bound FP2complex and the resulting FP2 was subjected to 100ns MD simulation. Similarly the holo state of FP2 bound with the inhibitor E64 was too prepared and subjected to 100 ns md simulation. This was followed by the analysis of the MD trajectories. The dynamics of the apo and the holo state of FP2 were visualized using discovery studio and PyMOL visualizing soft wares.

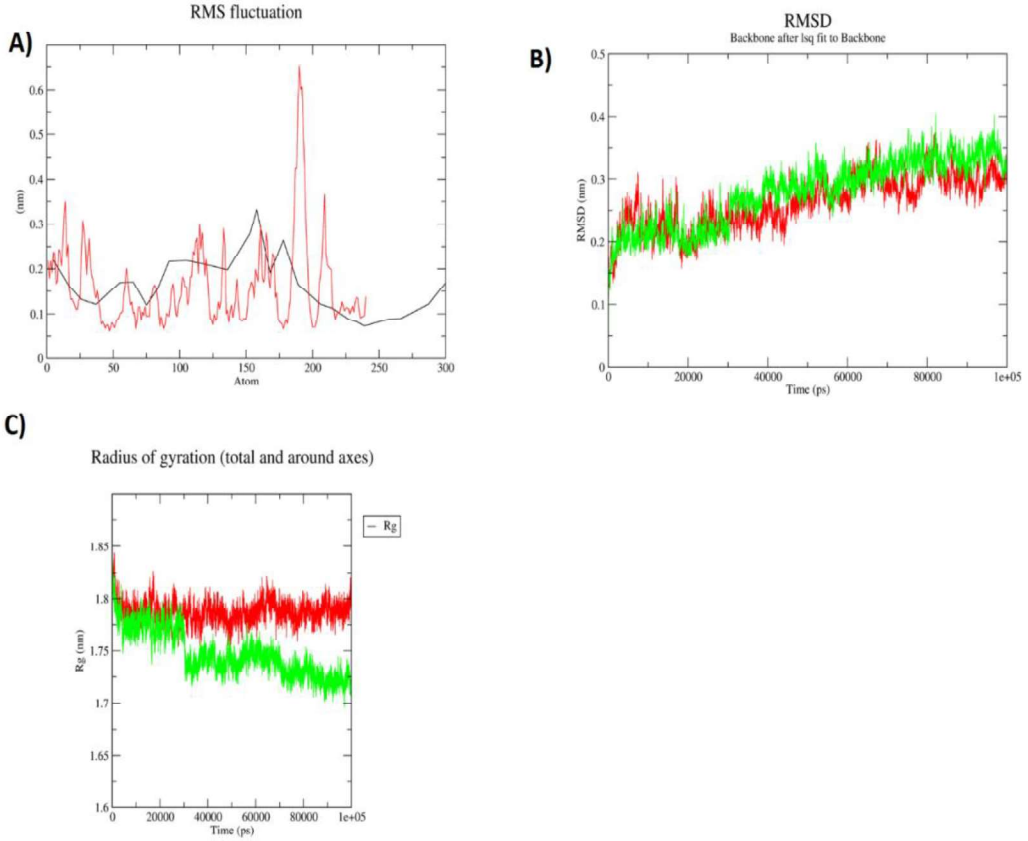
**MD simulation:**

MD simulations were carried using GROMACS package v4.5.5 [11]. The GROMOS 96–43a1 force field was employed for analyzing apo (without substrate) and holo (with substrate or inhibitors) structures. The Ligand topologies were generated using PRODRG2 server [12.]. In separate set of simulations, apo and holo protein complexes were placed in cubical boxes, equidistantly at 10Å distance from box edges. Following this, hydrogen atoms were added using pdb2gmx module of GROMACS and were constrained using LINCS algorithm [13]. With periodic boundary conditions applied in all three dimensions, protein was explicitly solvated using with SPC/E water model and appropriate counter ions were added to neutralize the system [14]. Solvated system was subjected to steepest descent energy minimization to remove steric conflicts between atoms. The energy minimized systems were then subjected to position restrained simulation in two different phases, NVT and NPT. Particle mesh Ewald (PME) method was employed to treat long range electrostatic interactions with cut off radius of 10Å.The system temperature of 300K was kept constant by modified Berendsen coupling. The structural coordinates were recorded at intervals for apo and holo state. The resulting trajectories were later analyzed for RMSD, Radius of gyration and Energy using XmGrace package to generate the 2D plot whereas PyMOL 1.3 (www.pymol.org) and DS Visualizer 3.5 were used for visual interaction analysis.

## Results:

To understand the stability of both apo and holo states, the RMSD of Cα backbone atoms was calculated. The calculated RMSD was found to be ~0.3 Å in holo state; whereas, the apo state had an average backbone deviation ranges of (0.25-0.35) Å. After initial 6 ns, the system remained in the plateau state till 100 ns (Figure 1B).Based on the intrinsic dynamics, the potential energy and total energy of the structure was calculated and the radius of gyration graph was plotted with respect to different time scale. As compared to the apo state, holo state had a decrease order of radius of gyration. The protein remained within compact radius of gyration of 1.7-1.75 Å, indicating stable nature of the holo state of the protein (Figure 1C). RMSFs of Cα atoms of the holo structure showed similar type fluctuations throughout the simulation process with minute deviation at the N-terminal end than the apo state. As evidenced from (Figure 1A), it can be clearly observed that the amino acids positioned in 150–300 (within the catalytic domain) residues showed more deviation reflecting flexibility of this region in protein. In addition, energy profile

of the both systems i.e., the kinetic and potential energy of apo as compared the holo state indicated the dynamic stability of the ligand bound holo state (Figure 1 D&E)



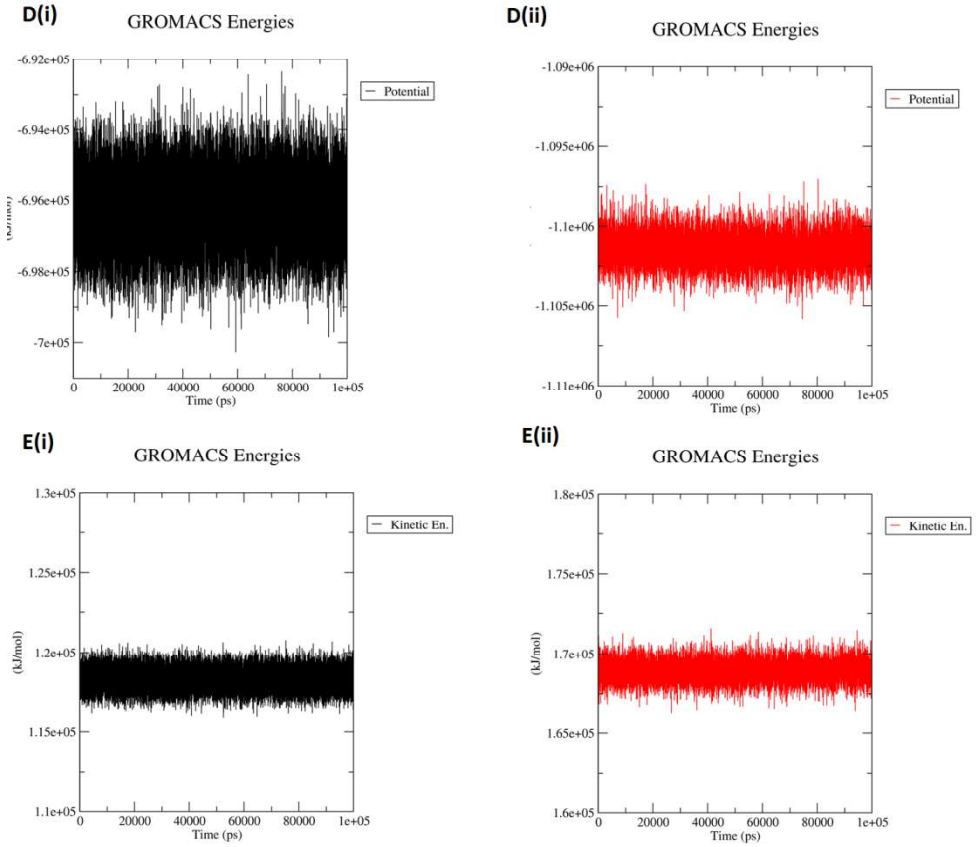
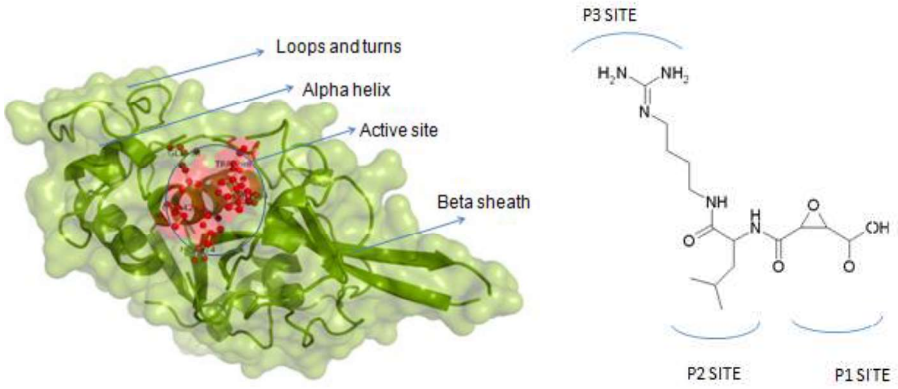



Fig1: The RMSD, RMSF and radius of gyration graph of FP2-E64 during MD simulation. (A) RMSD of backbone Cα atoms of FP2 in apo and holo state. (B) RMSF analysis of amino acid residues of FP2 in apo and holo state. (C) Radius of gyration of FP2 in apo and holo state. (D) Potential Energy. (E) Kinetic energy. All the images were generated using XMGRACE software. Green/ black and red colors represent the holo and apo state, respectively.

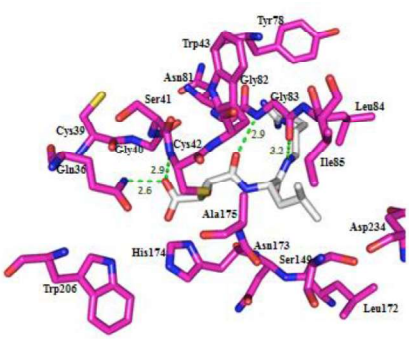
## Discussion:

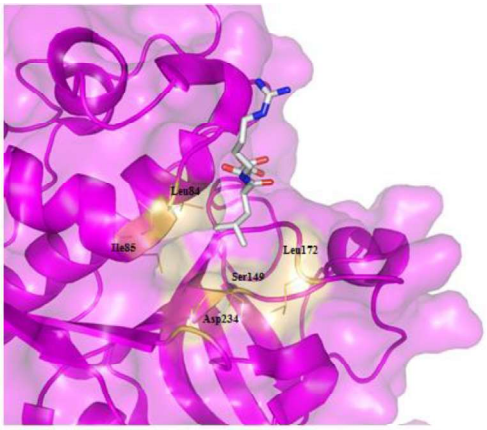
Enzymes lower the Activation Energy of the chemical reaction and bring the substrate to the active sites in a suitable orientation for an enzyme- substrate complex, which often involve rearrangement of atom in the active sites. Ligand binding models propose that binding of a substrate or an inhibitor in the active sites would be accompanied by conformational changes. Indeed different conformations have been observed for apo and holo protein in experimental and computational studies.

In this work, we present a comparative analysis of apo and holo structural state of FP2 with its inhibitor E64.In the absence of E64, MD simulations of its apo form provided preliminary insights on its 3D conformation. The active sites of the enzyme is located in a cleft between the structurally distinct domains of the papain-like fold. E64 interact with residues in the S1, S2, and S3 subsites of FP2, corresponding to the P1, P2, and P3 positions of the ligand. The conserved catalytic residues of FP2 (Gln36, Cys42, His174, Asn204) are similarly oriented with respect to the inhibitor E64. The active site cysteine forms a covalent, irreversible hemithioketal with the E64 epoxy carbon in the FP2-E64 complex The peptidyl small molecule inhibitors are tethered to the main chains of FP2 through a glycine residue that is highly conserved in the S3 subsite of clan CA cysteine proteases(Gly83) (Figure 2 A-D) [15]. In the complex, this residue forms hydrogen bonds with the O and N atoms of the inhibitor backbone (Figure 2E- F). In the FP2 active site, Gln36, Ser41 (monomer C only), Cys42, Asn81 (monomer A only), and His174 are involved in the formation of additional hydrogen bonds with E64 [16]

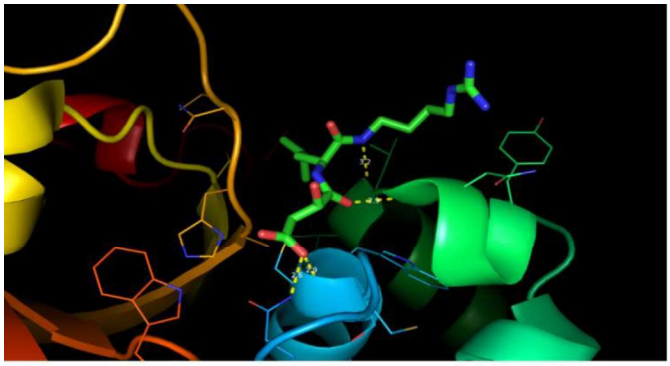


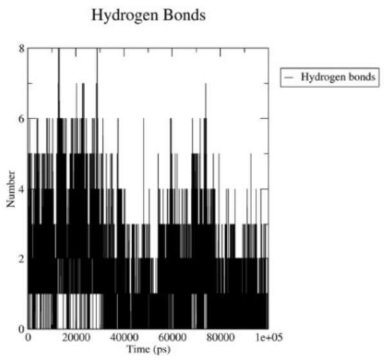

(A) (B)




(C) (D)



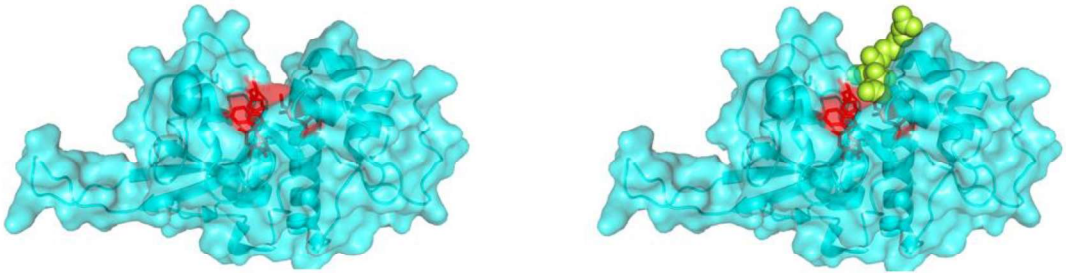



(E) (F)

Fig2: (A) Overall view of Falcipain 2. a ribbon and surface representation of Falcipain 2 with catalytic residues(cys42,gln36,asn204,his174,trp206) encircled at the centre in ball-and-stick models. (B) Figure represents the inhibitor E64. (C) Active sites of FP2 (3BPF). FP2-E64 complex. Important residues in the active site are colored pink and labeled. E64 is colored gray, and interactions with the enzyme are in green. (D) Surface representations of FP2 highlighting the contour of the S2 subsite and important residues therein. Ligand E64 is depicted in grey. (E) Representing the ligand (E64) binding sites with FP2. The yellow lines represent the h-bonds with distance. (F) Graph depicts the hydrogen bonds between E64 and Falcipain 2.

The structural comparison of apo and the holo state of various enzyme highlights that, the main structural difference between the two states is the different conformational changes of active sites residues. At the unbounded state, the active

cleft is left open accessible to substrate. Whereas in the bound state the cleft adopt a closed conformation which block the entry to the active sites. Compared to un complexed enzyme ligand binding induces a more compact conformation enclosing more tightly specific subsites of the inhibitor and inducing more effective inhibition. (Figure 3) The trapping of the ligand into cleft shields the ligand molecule fully or partially from aqueous environment thereby stabilizing the bond state. Moreover access of the active sites to other molecules is prevented and the reaction intermediates are protected and stabilized [17]. This mechanism provides an effective way to control the accessibility of the substrate to the active sites residues, thereby inhibiting the enzyme function.



(A) (B)

Fig 3: (A) represent the apo structural state if FP2 with the active sites residues colored as red. (B) Fig represents the holo structural state of FP2-E64 complex. Important residues in the active sites are colored red and the ligand E64 is shown in green spheres.

The 100 ns simulation of the apo and the holo form of the enzyme stabilizes from 80ns onwards (Figure1B). Moreover stability of the enzyme in apo and holo state was confirmed by the consistency of the potential and kinetic energy graphs (Figure1 D&E). Apart from this the MD trajectories for RMSD, RMSF, Radius of gyration also inferred that binding of the ligand(E64) leads to a more compact and stable conformations of the protein-ligand complex inducing more effective inhibition which a prerequite to the design of future anti-malaria drugs targeting FP-2.

## Conclusion:

On analyzing the MD trajectories and overall structural and conformational dynamics, it may be concluded that binding of the ligand leads to a more compact and stable conformations of the protein-ligand (FP2-E64) complex inducing more

effective inhibition. Further, since small molecule inhibitors like E64 are much more attractive as potential leads for the treatment of malaria and more bioavailable, amenable to oral or topical administration, also vastly easier to redesign. Therefore, such holo structure with ligand-enzyme interaction plays a major role in drug discovery process, and novel computation methods can further apply these informations to model new unknown interactions and therefore contribute to answer, open questions in the field of drug discovery like ligand 3d homology, induce fit simulations etc.

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