

Integrated High-Throughput Virtual Screening and Molecular Dynamics Identify Promising *Plasmodium falciparum* D-Enoyl-ACP Reductase Inhibitors for Drug Discovery

Stephenson Obeng Owusu¹, Prince Manu¹, Muntawakilu Padiga Seidu¹, Alice Adomako¹, Ophelia Duodu¹, Philip Thompson¹, Yvonne Adjei Kankam¹, Michael Konney Laryea¹ and Lawrence Sheringham Borquaye^{1,2}

¹Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

²Central Laboratory, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

1. INTRODUCTION

Malaria remains a major public health issue worldwide and a repeated cause of illness and death in tropical and subtropical areas. Despite decades of intervention efforts, millions of new cases and hundreds of thousands of death still occurs each year, primarily in low and middle-income countries [1]. As one of the deadliest infectious diseases known to man, the evolution of resistance in malaria parasites underscores a the critical need to the development of novel antimalarial agents with unique mechanism of actions[2].

The study aims to identify potential inhibitors of *P.falciparum* D-enoyl-ACP reductase (PfENR) through integrated high-throughput virtual screening and MD simulations, with the goal of discovering promising lead compounds for antimalarial drug development.

2. EXPERIMENTAL DESIGN

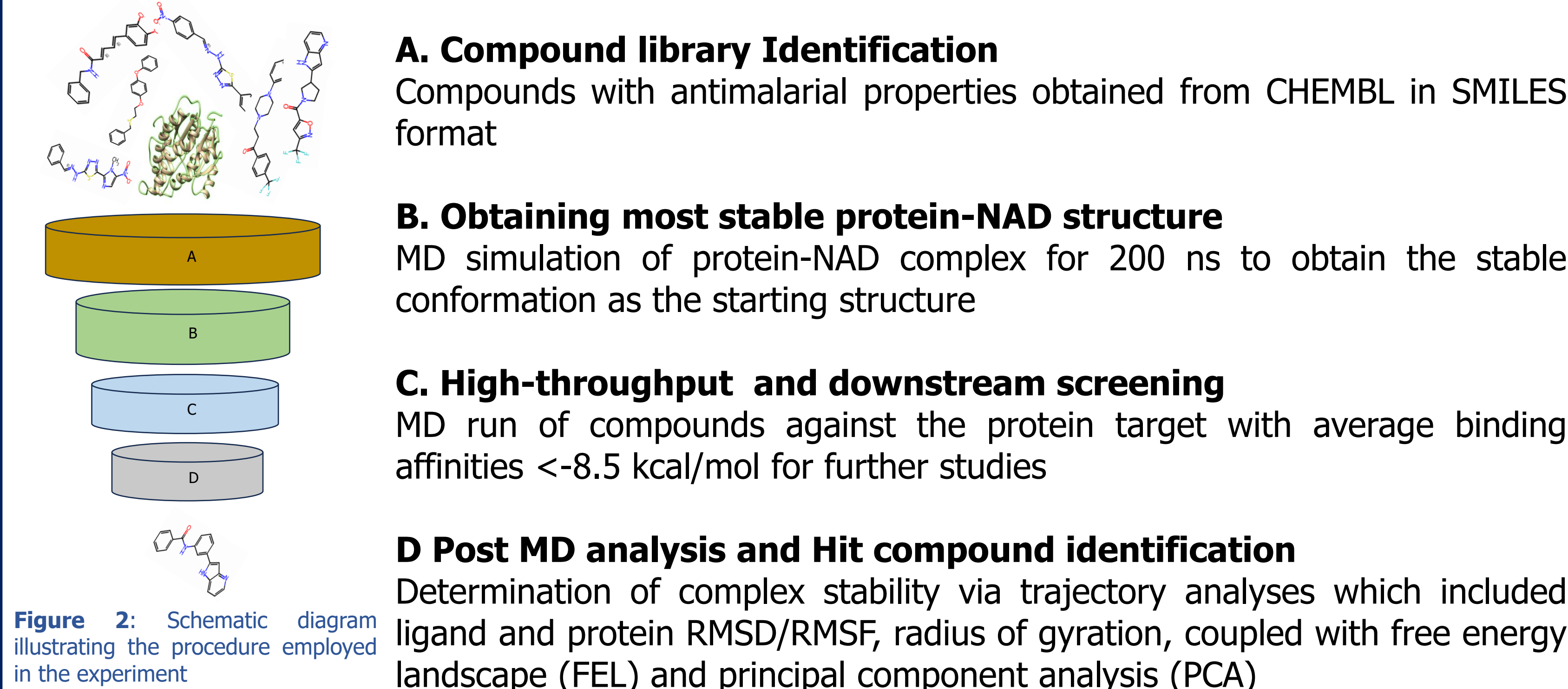


Figure 2: Schematic diagram illustrating the procedure employed in the experiment

3. RESULTS

i Three-dimensional structure of the PfENR tetramer

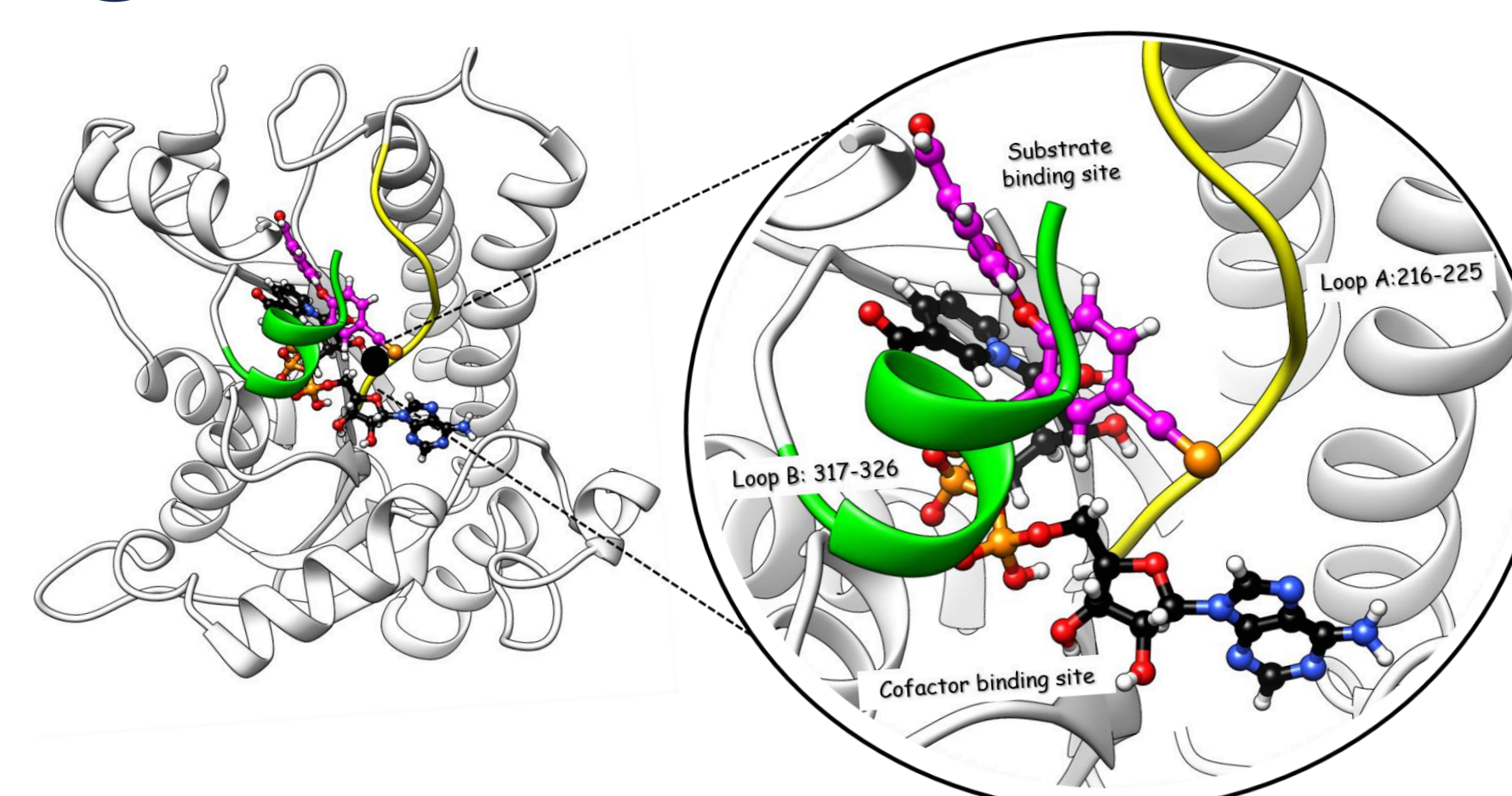


Figure 3: Two monomers are shown in cartoon representation, highlighting the overall ternary architecture. The NADH cofactor occupies the cofactor-binding pocket, while the inhibitor interacts with residues within the substrate-binding region. Both ligands are displayed in ball-and-stick representation. Flexible active-site loops surrounding the binding pocket are also evident, suggesting their potential role in ligand recognition and binding.

ii Root Mean Square Deviation (RMSD) analysis of ligand-protein complexes

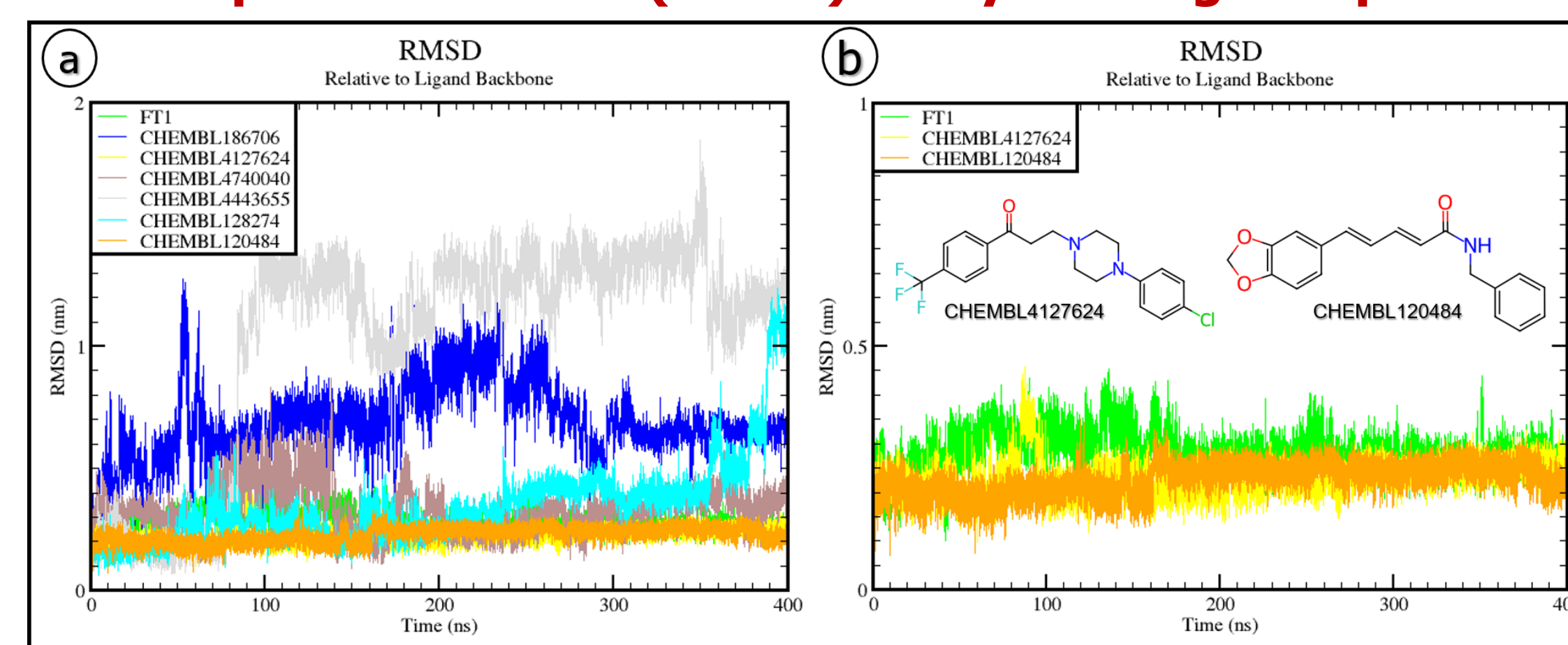


Figure 4: (A) RMSD profiles calculated relative to the ligand backbone indicate that the selected compounds maintain stable trajectories throughout the 500 ns molecular dynamics simulations. (B) The top-performing ligands, CHEMBL4127624 and CHEMBL120484 exhibit stable conformational behaviour, with RMSD values remaining below 0.25 nm, suggesting sustained binding stability and minimal structural fluctuations during the simulation period.

iii Dynamics of CHEMBL4127624-NAD interactions at the protein active site

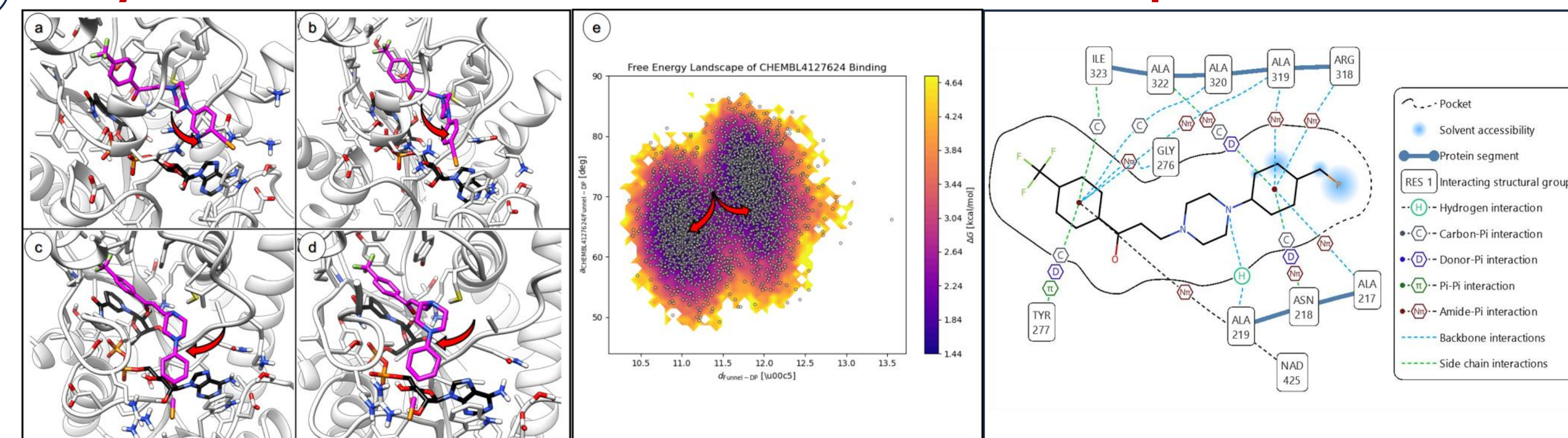


Figure 5: (A) Time-resolved molecular dynamics snapshots illustrate the progressive interaction between the ligand and the nicotinamide ring of NAD. Panels a and b show the ligand approaching NAD, while panels c and d demonstrate the establishment of n-n stacking interactions and associated conformational transitions. These observations align with (E) the free-energy landscape, indicating a favourable and stable ligand-binding state.

iv Dynamics of CHEMBL120484-NAD interactions at the protein active site

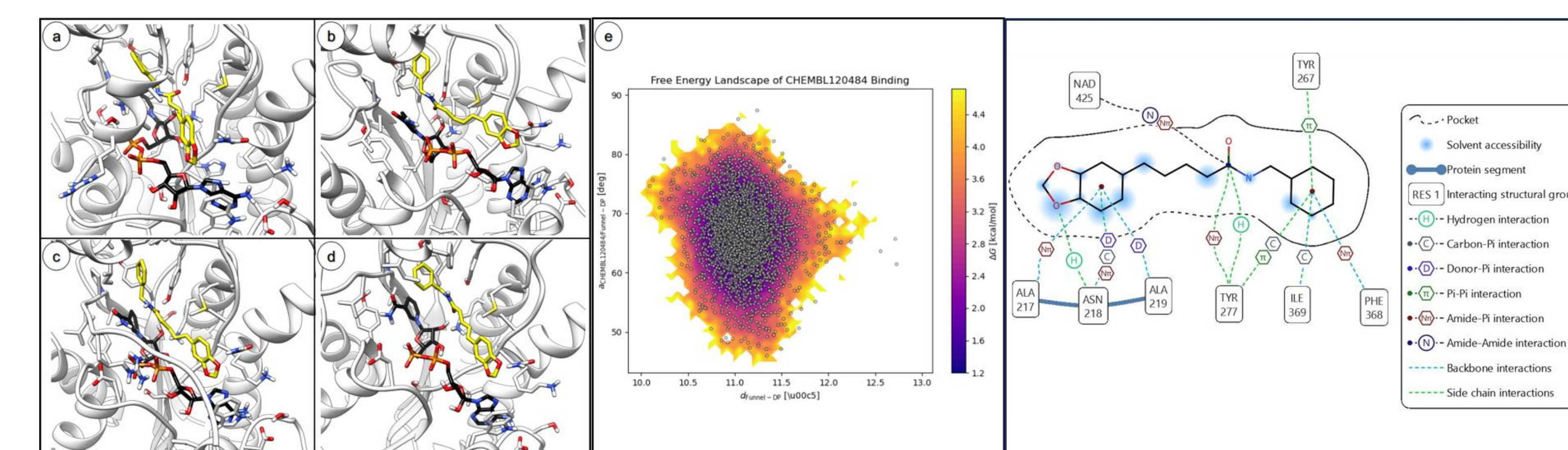


Figure 6: (A) Molecular dynamics snapshots illustrate the progressive association of the ligand with the nicotinamide moiety of NAD. Panels a and b show the ligand approaching the cofactor, while panels c and d depict the formation of strong interactions accompanied by moderate conformational adjustments. These changes are consistent with (E) the free-energy landscape and indicate stabilization of the ligand-NAD complex.

v NAD (cofactor) dynamics at the protein active site in the presence of a ligand

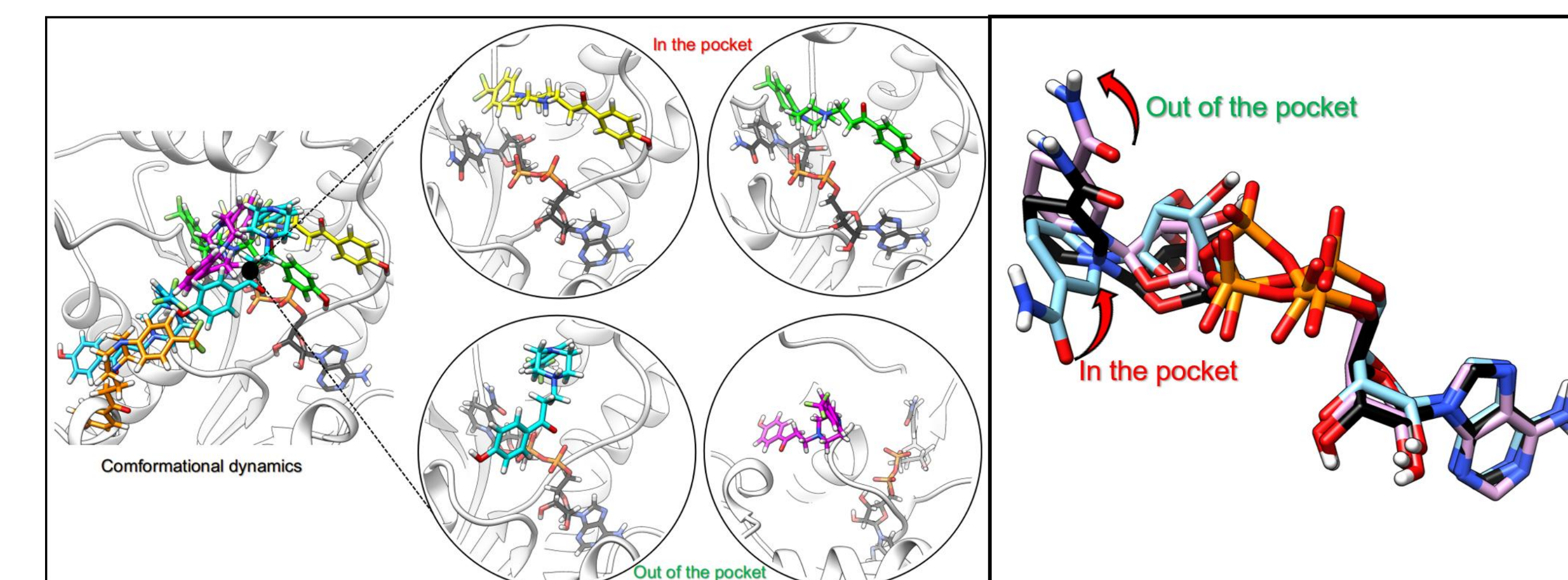


Figure 7: (A) Structural superimposition reveals an outward flipping of the nicotinamide ring of NAD in the absence of a ligand. (B) In contrast, ligand binding stabilizes an inward-facing conformation of the cofactor, promoting favourable interactions between NAD and the ligand. These conformational differences support the hypothesis that ligand-mediated NAD interactions play a key role in modulating protein activation and inhibition.

4. CONCLUSION

MD simulations and structural analyses indicate that CHEMBL4127624 and CHEMBL120484 form stable complexes with PfENR and promote favourable interactions with the NAD cofactor. The observed ligand-induced changes in NAD orientation suggest a cooperative protein regulating mechanism, highlighting the potential of these compounds as inhibitors to PfENR.

[1] Alharbi BF, Ahmed MA. Malaria in the 21st Century: Global Disease Burden, Epidemiological Insights, and Strategic Control Approaches. *Biology*. 2026 Apr 3;15(7):575.
[2] Manu P, Padiga Seidu M, Adomako A, Asamoah CK, Duodu O, Britwum A, Laryea MK, Darko G, Borquaye LS. Computational elucidation of Plasmodium falciparum enoyl-ACP reductase inhibition by Salacia debilis compounds: adaptive ligand binding drives antimalarial activity. *In Silico Pharmacology*. 2025 Aug 27;13(3):121.