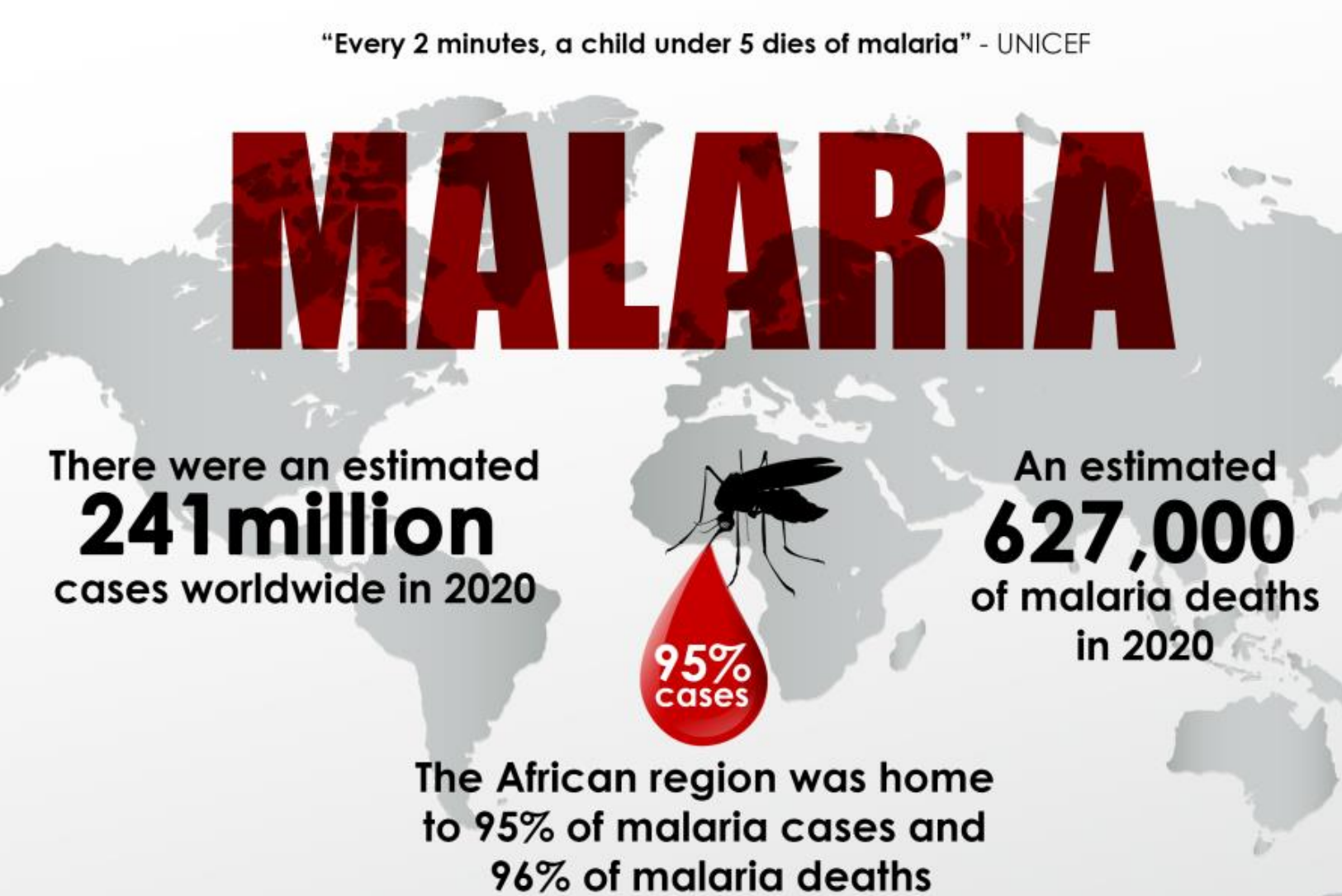


1 Introduction

Malaria remains a global health emergency and causes millions of infections and thousands of deaths annually, concentrated in low and middle-income countries. UNICEF reports that in "every 2 minutes, a child under 5 dies of malaria", underscoring the urgent need for viable and novel alternatives to the failing therapeutics[1].



Malaria burden stretches beyond illness to poverty, strains health systems and undermines development across endemic regions.

Natural products including *Salacia debilis* (SD) are among native plants with potent antimalaria activity. While Laryea and colleges isolated and established the antimalarial activity of SD isolates[2], understanding their protein targets and possible mechanisms of action is crucial for drug optimization and development.

This study employ target fishing, high-throughput screening, and homology screening to identify the PfENR as a possible target for the isolated compounds and proposed a possible mechanism.

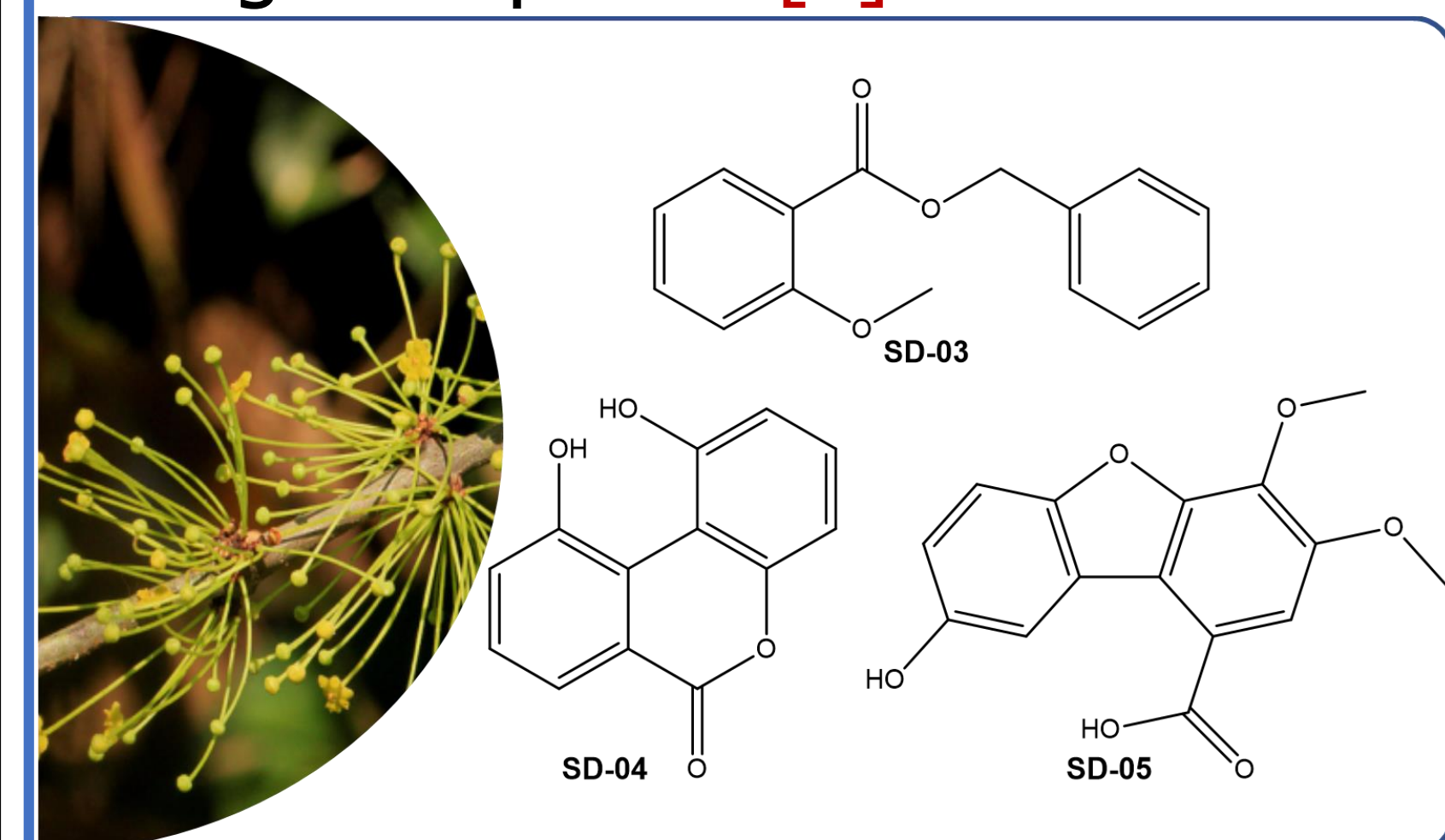


Figure 1: *Salacia debilis* and its isolated compounds

2 Experimental Design

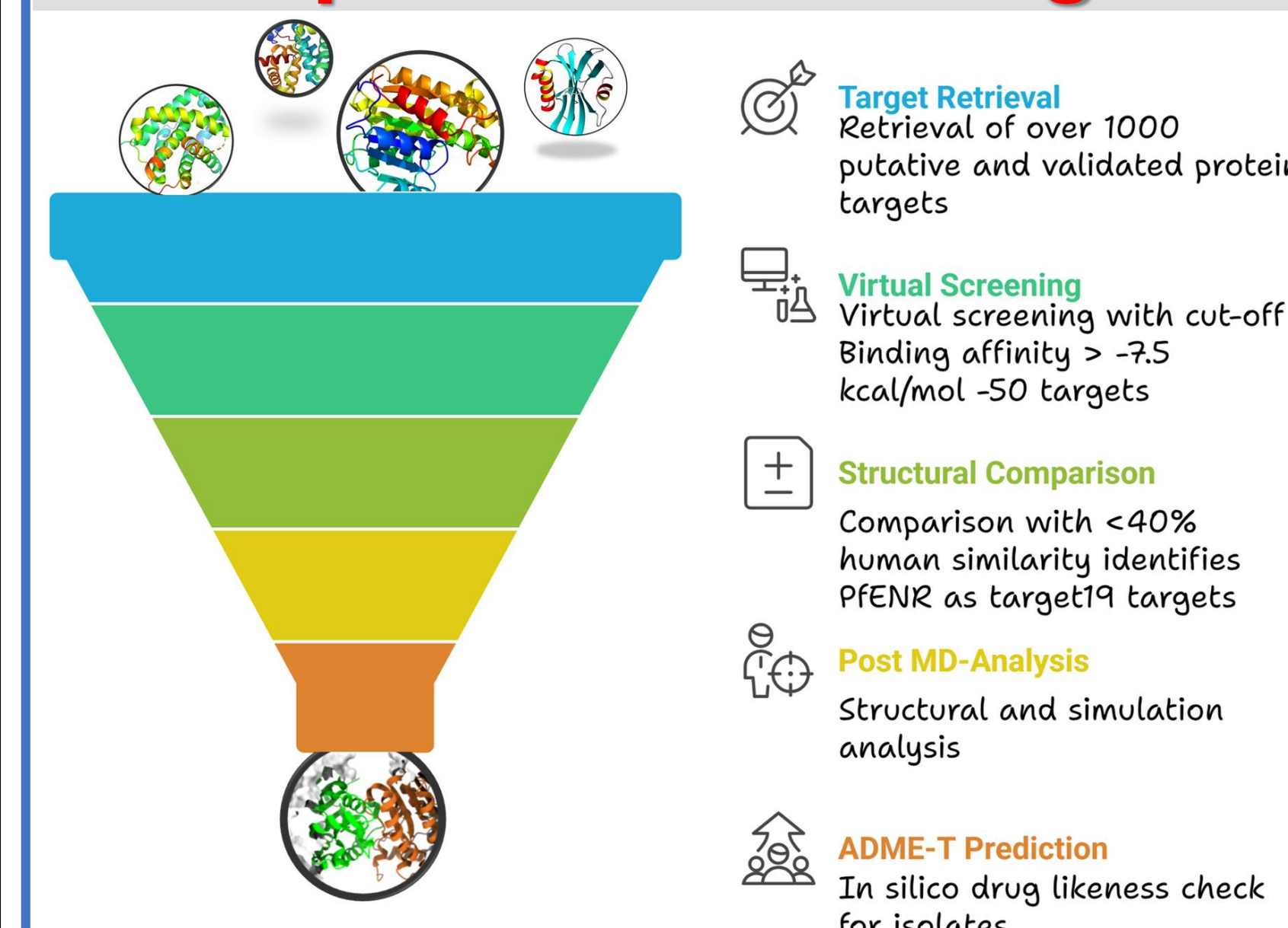


Figure 2: Scheme of the experiment illustrating the systematic procedure employed in the experiment

3 Results

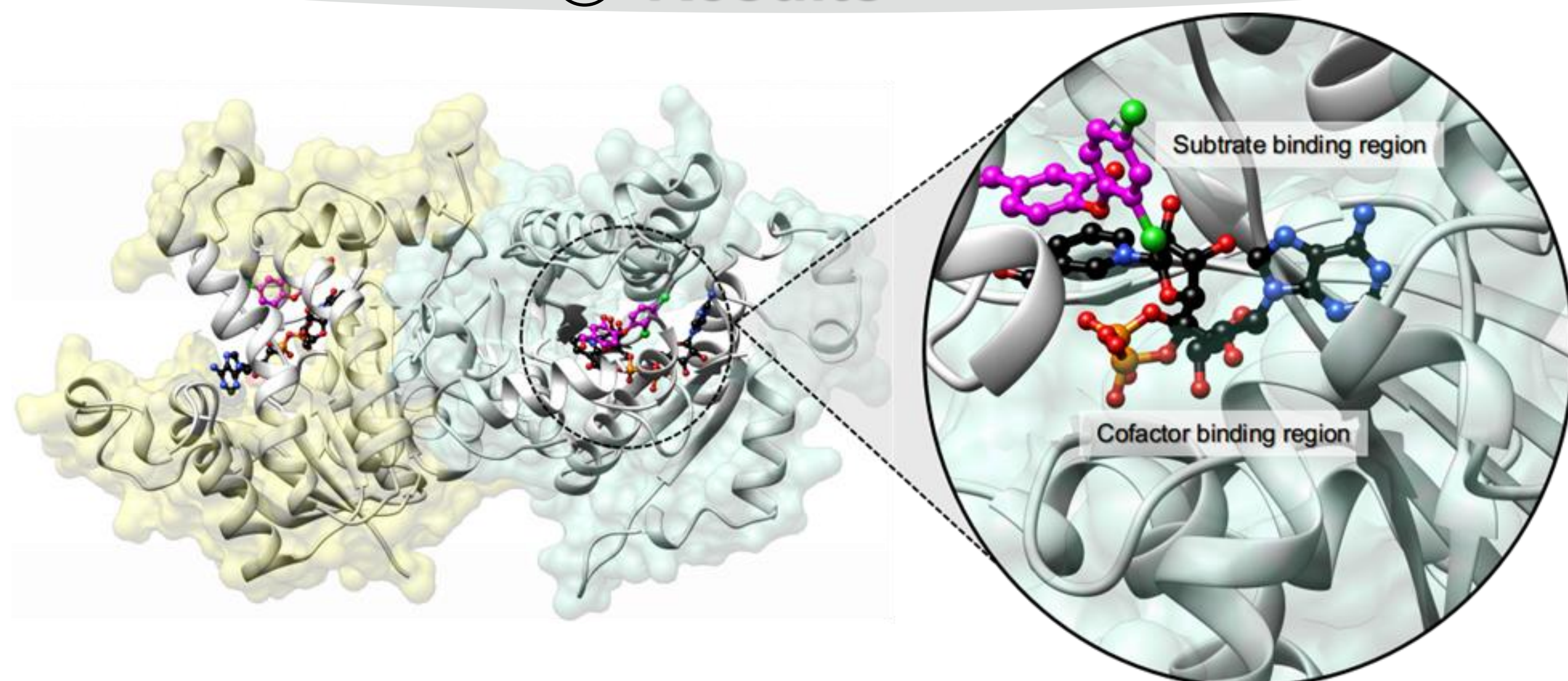


Figure 3: Three-dimensional structure of *Plasmodium falciparum* enoyl acyl-carrier protein reductase (PfENR) tetramer, with two monomers shown in cartoon representation. The NADH cofactor (black) occupies the Rossmann-fold pocket, while the inhibitor triclosan (magenta) resides in the substrate-binding region. Both ligands are depicted in ball-and-stick and are cradled by the flexible active-site loops.

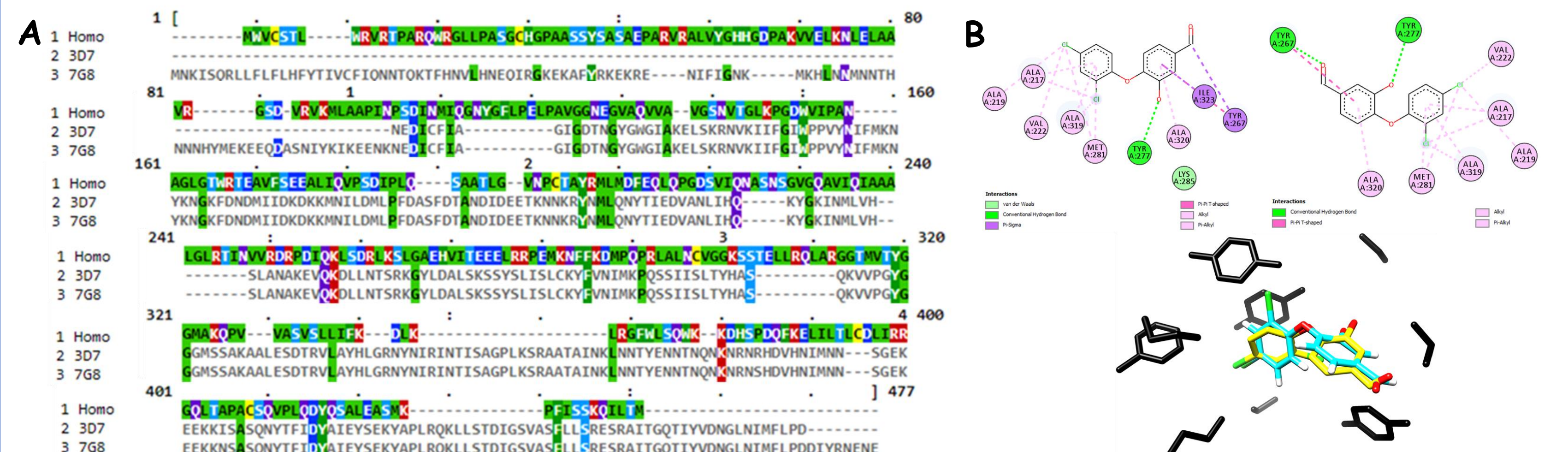


Figure 4: A - Multiple sequence alignment of *Plasmodium falciparum* enoyl acyl carrier protein reductase (PfENR) with its human homologs. This figure shows the sequence alignment of PfENR (3D7) compared against human enoyl-acyl carrier protein reductase (labeled "Homo") and a closely related structure (7G8). Conserved residues are highlighted with color codes: green for small or hydrophobic residues. B - Method validation by superimposition of docked and crystal structure orientation of Triclosan showing RMSD of 1.225 Å

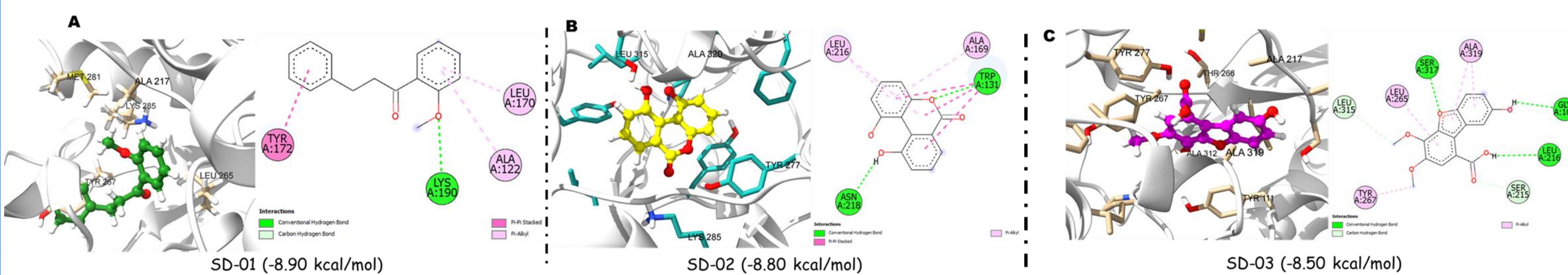


Figure 5: Molecular docking results showing the binding interactions of selected compounds with PfENR. Panels A, B, and C represent compounds SD-03, SD-04, and SD-05, respectively. For each compound, the left image illustrates the 3D interaction map, while the right image displays the corresponding 2D binding pose within the active site of PfENR. These visualizations highlight key interactions such as hydrogen bonds, hydrophobic contacts, and positioning within the binding pocket

4

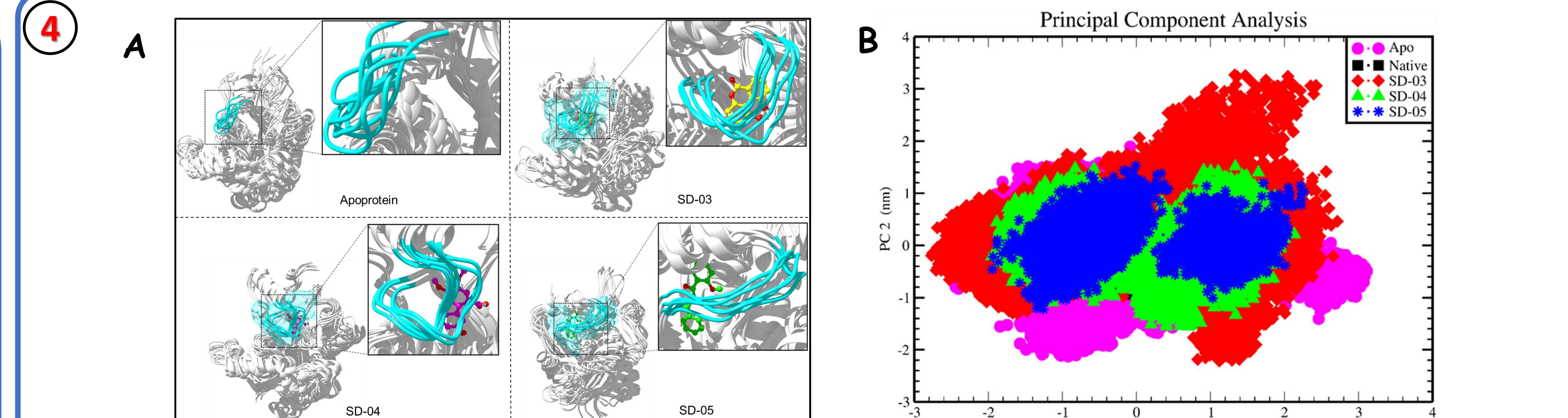


Figure 6: A - Cluster of average protein structural conformations throughout the simulations at 100 ns intervals showing structural conformations of the protein active site loops. B - Principal Component Analysis (PCA) of the apo and bound PfENR complexes shows major conformational dynamics and clusters of the protein in its bound and unbound (apo) form and when bound to the SD compounds.

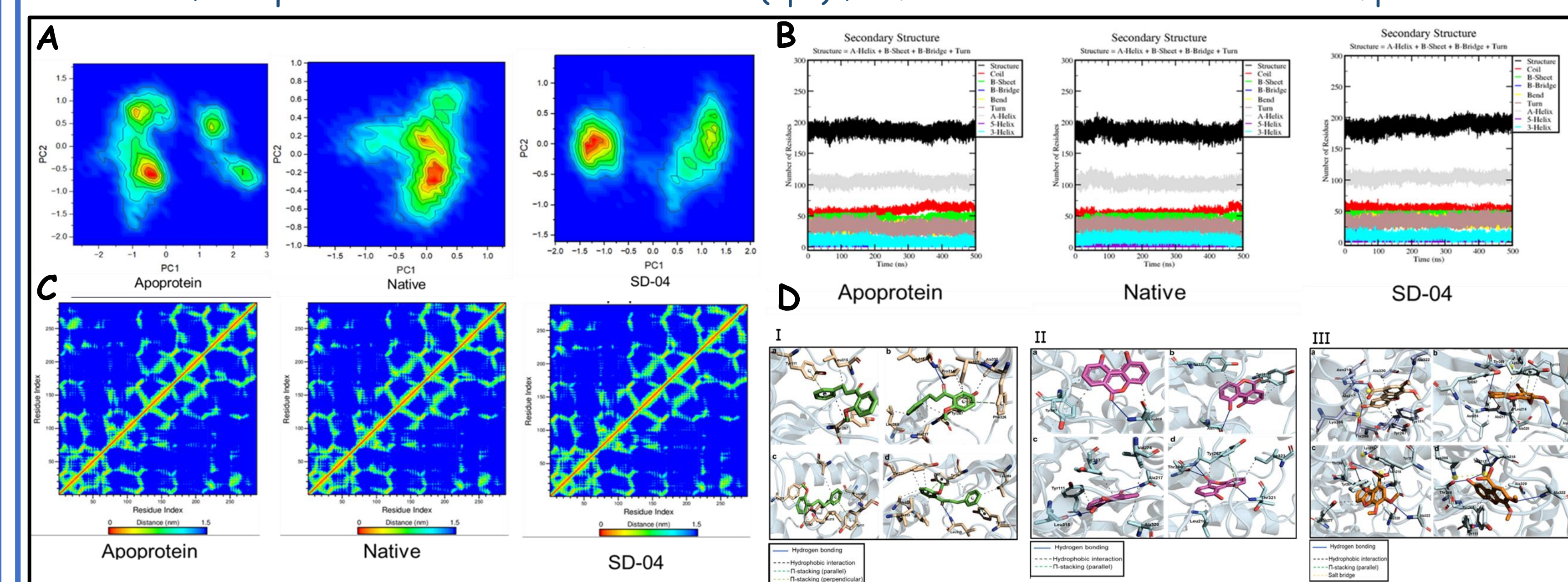


Figure 7: (A)-FEL of principal components 1 and 3. (B)-DSSP shows the secondary structure dynamics of the apo and bound complexes, highlighting structural changes over the simulation period. (C)-DCCM illustrates residue-residue motion correlations in the complexes. (D)-Binding interactions of SD-03 (I), SD-04 (II), and SD-05 (III) within the PfENR active site at 100 ns (a), 250 ns (b), 350 ns (c), and 500 ns (d) of molecular dynamics

5 Conclusion

The study identified *Plasmodium falciparum* enoyl-acyl carrier protein reductase (PfENR) an enzyme essential for parasite fatty acid biosynthesis but absent in humans as the molecular target of *Salacia debilis* isolates.

Among the isolates, SD 04 emerged as the lead candidate based on favorable pharmacokinetic predictions.

[1] Malaria in Africa - UNICEF DATA
[2] Laryea, M.K. et al. (2023) 'Antimalarial compounds from the climbing stems of *Salacia debilis*', *Natural Product Research*, 0(0), pp. 1-10. Available at: <https://doi.org/10.1080/14786419.2023.2272288>
[3] Maity, K., Bhargava, S. P., Sankaran, B., Surulia, N., Surulia, A., & Suguna, K. (2010). X-ray crystallographic analysis of the complexes of enoyl acyl carrier protein reductase of *Plasmodium falciparum* with triclosan variants to elucidate the importance of different functional groups in enzyme inhibition. *IUBMB Life*, 62(6), 467-476. <https://doi.org/10.1002/iub.327>