# Molecular modeling study in solubility improvement of *Plasmodium falciparum* dihydrofolate reductase by rationally designed amino acid replacement


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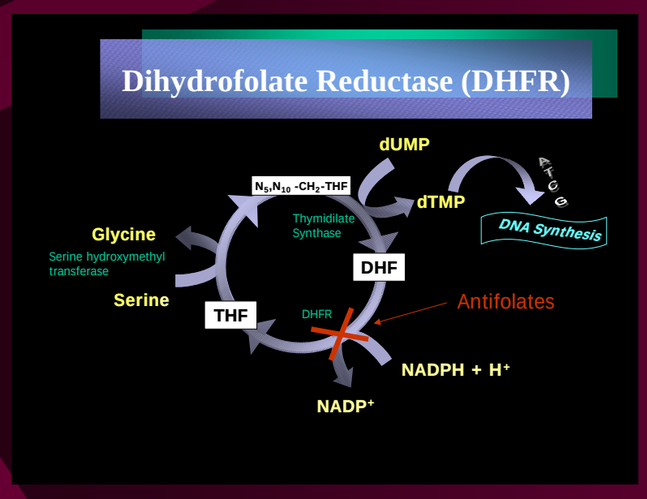
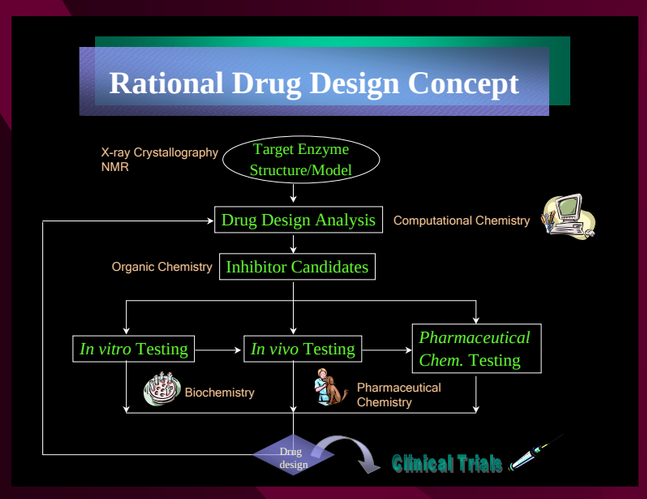
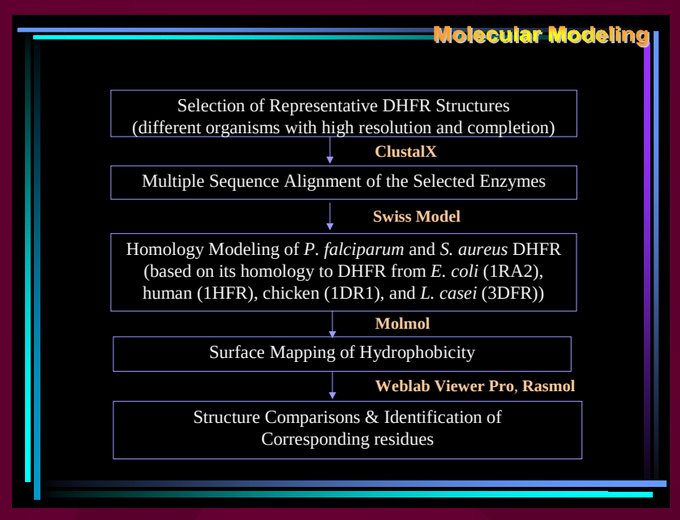
## Abstract

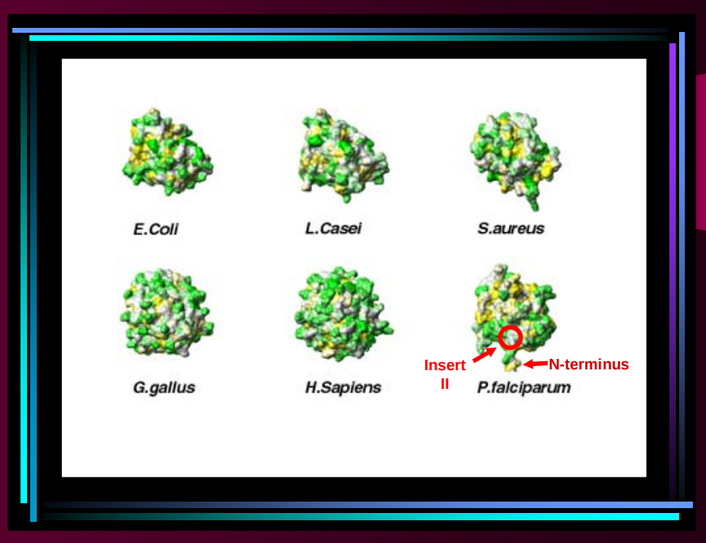

Malaria is a widespread, tropical disease among many developing countries. A severe mortality rate and the emergence of highly drug-resistant protozoa *Plasmodium falciparum* have placed antimalarial drug development in a very critical situation. One aspect of the research in this field is the design of inhibitors for *P. falciparum* dihydrofolate reductase (*Pf*-DHFR) since inhibition of this key enzyme can lead to the parasite's death because of the depletion of tetrahydrofolate essential for the synthesis of thymidylate and hence DNA. However, the extremely low solubility of *Pf*-DHFR has prevented structure determination of this enzyme either by X–ray crystallography or NMR spectroscopy. The lack of three-dimensional structure, in turn, has limited the utility of rational design of *Pf*-DHFR inhibitors. This project, therefore, aimed to identify candidate amino acids on the surface of *Pf*-DHFR that might affect its solubility using molecular modeling techniques and comparison with model structure of *Staphylococcus aureus* DHFR type S1. In addition, sizes of polypeptides resulted from limited tryptic digestions of *Pf*-DHFR as estimated by 15% SDS-PAGE suggested the positions of big inserts were placed correctly in the homology model of *Pf*-DHFR. This assured the reliability of the model core structure positioning for such purpose. We propose these residues include F37, F116, L131, I150, V151, L152, L153, L156, and I200.

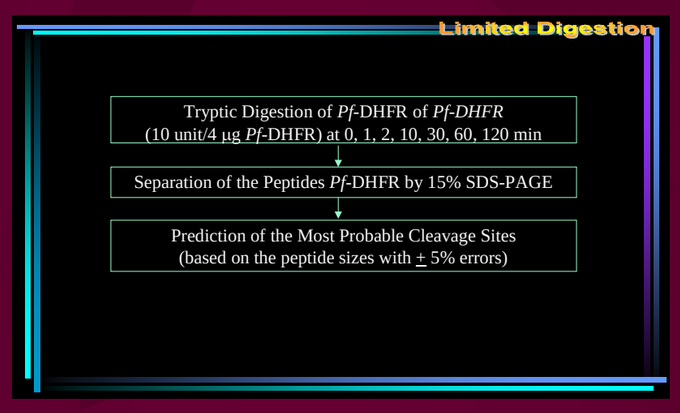



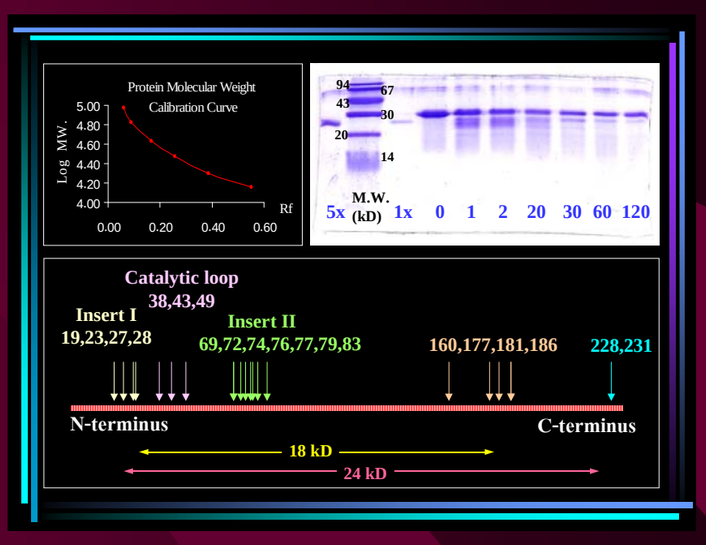




## Methodology



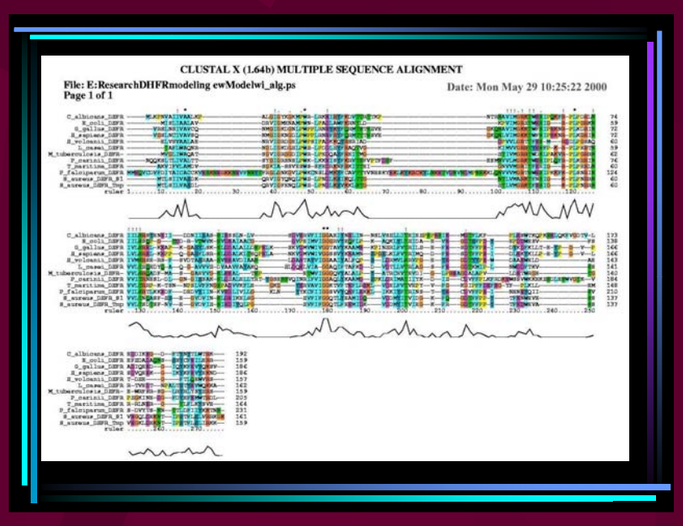



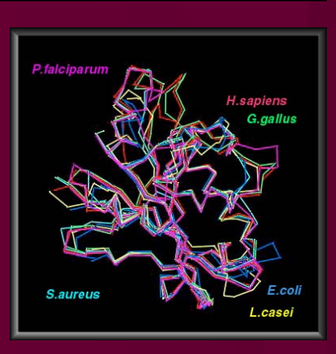




## Results & Discussion

The multiple sequence alignment showed that *Pf*-DHFR has two extra insertions of 16 and 34 residues with respect to the other species with available structures. As there is no structural information on these long inserts, the *Pf*-DHFR sequence without the inserts was used to obtain the *Pf*-DHFR model.

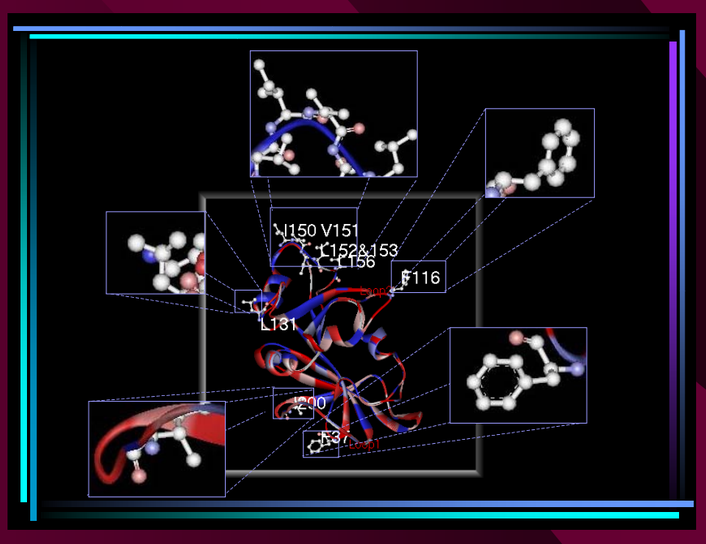







Since mutations of hydrophobic surface residues of *Sa*-DHFR to polar amino acids could increase its solubility (Dale, *et. al.*), the models of *Sa*-DHFR type S1 and *Pf*-DHFR without the inserts were compared to identify candidate residues for similar mutations of *Pf*-DHFR. However, all the amino acids corresponding to the mutated *Sa*-DHFR residues were already hydrophilic in the *Pf*-DHFR.

As the lack of information about the two inserts can complicate the model building and the prediction, the tryptic digestion of *Pf*-DHFR was done to confirm its reliability. Since the more flexible, water exposed residues in the turn region of the protein would be digested faster, the time-coursed, limited tryptic digestion of *Pf*-DHFR was introduced as an independent probe. All of the most probable cleavage sites predicted with no structural knowledge were found in the exposed loop regions of the model, indicating some accuracy of the model.




Other bulky hydrophobic residues on the *Pf*-DHFR surface away from its active site that could affect solubility but not catalytic properties were located: F37, F116, L131, I150, V151, L152, L153, L156, and I200.


## Acknowledgement:

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## References:


(1) Dale, G. E.; Broger, C.; Langen, H.; D'Arcy, A.; Stuber, D. *Protein Engineering* 1994, 7: 933–939.
(2) Duangrudee Tanramluk, Jirundon Yuvaniyama, Yongyuth Yuthavong, *KU Science J.*, 2001, 19(1–3), 64–73