In-Silico Structural and Functional Analysis of Cytochrome P450 Enzyme in *Aedes aegypti*

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ABSTRACT

Cytochrome P450 (CYP450 or CYPs) enzymes play a crucial role in metabolic adaptation and detoxification in insects, particularly from harmful chemicals and insecticides. This study analyzes the CYP450 Enzyme from *Aedes aegypti,* a mosquito species known for transmitting dengue, Zika, and chikungunya viruses. The enzyme's amino acid sequence was retrieved from UniProt, and its 3D structure was modeled using Swiss-Model. The 3D structure was visualized in PrankWeb (P2Rank) to predict its active or binding sites in the protein. Understanding Cytochrome P450, a detoxifying enzyme, can help uncover the mechanism of insecticide resistance which poses a major challenge in vector control strategies.

INTRODUCTION

Cytochrome P450 enzymes are a large family of enzymes found in almost all living organisms. They are like tiny molecular machine that helps maintain balance and protect life. In insects, they help in breaking down toxins and contribute to the development of insecticide resistance. CYP enzyme is a heme group containing iron and allows interaction of enzymes with oxygen. This interaction is necessary because CYPs use oxygen for modification of other molecules. The process of modification is carried by the addition of one atom to a substance while converting the other into water; this process is called monooxygenation. Besides insects, these enzymes are found in fungi, bacteria, plants, and animals, and are essential for detoxification, metabolism, and hormone synthesis.

MATERIALS AND METHODS

Steps	Tools	Action
1. Sequence Retrieval		The amino acid sequence of CYP450 from *Aedes aegypti*

	UniProt Knowledgebase (The UniProt Consortium, 2025)	was downloaded in FASTA format
2. 3D structure modeling	Swiss-Model (Waterhouse *et al.*, 2018)	The sequence in FASTA format was used to build a 3D model
3. Active Site Prediction	PrankWeb (P2Rank) web server (Polák *et al.*, 2025)	The selected model was uploaded for visualization and to predict pockets or binding Sites.

RESULT AND DISCUSSION

The 3D structure of Cytochrome P450 enzyme from *Aedes aegypti* (UniProt ID: A0A6I8TSY) was successfully modeled using Swiss-model and PrankWeb. The predicted CYP450 showed a globular shape, composed mainly of alpha-helices scattered with short beta-strands, which is a typical structural characteristic of CYP450 enzymes.

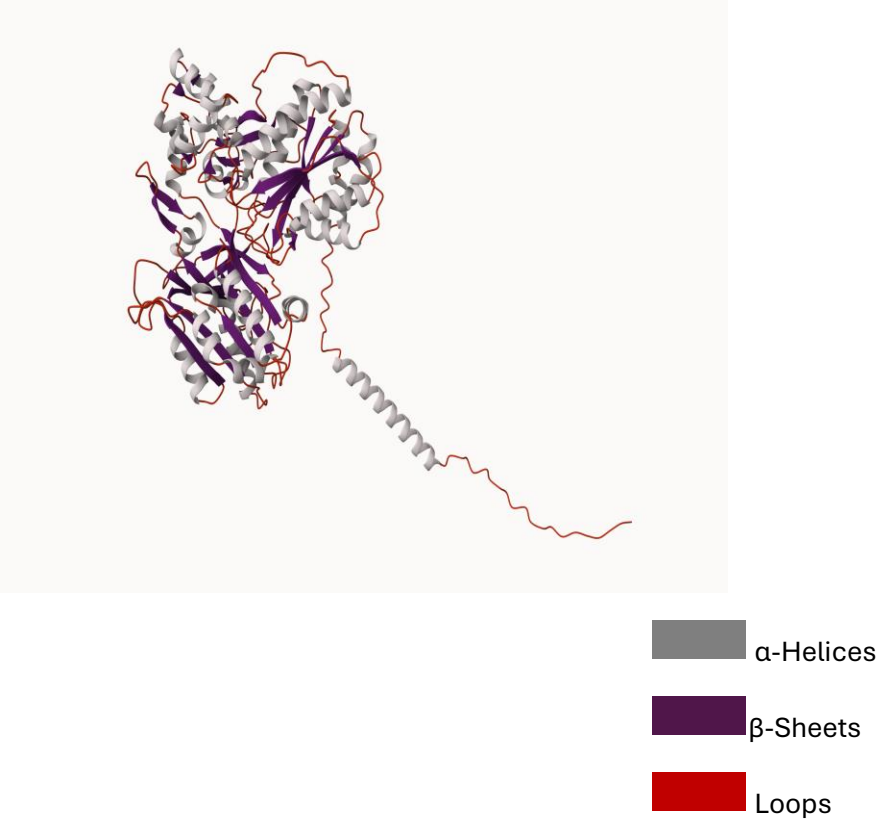


Figure 1: 3D structure of Cytochrome P450 enzyme displaying α-helices (green), β-sheets (yellow), and loops (red) for visual reference before active site prediction.

Swiss-model created four structural models of *Aedes aegypti* Cytochrome P450. Model 01 (template: U5EZ84.1.A, NADPH–cytochrome P450 reductase) showed the highest sequence identity (84%) and highest GMQE (0.91), therefore it was chosen as the final Model. Models 02–04 had lower GMQE and QMEAN scores, hence it was considered less reliable.

Table 1. Comparison of Cytochrome P450 models generated by Swiss-model

Model	Template (PDB ID)	Sequence identity (%)	GMQE	QMEANDisCo Global	Remarks
Model 01	U5EZ84.1.A	84.09%	0.91	AlphaFold-derived template	Selected model; highest quality and identity
Model 02	4yaf.1.A	59.31%	0.79	0.81±0.05	Moderate quality
Model 03	6j7a.1.A	58.14%	0.79	0.79±0.05	Low quality
Model 04	3fjo.1.A	50.25%	0.75	0.77±0.05	Lowest quality

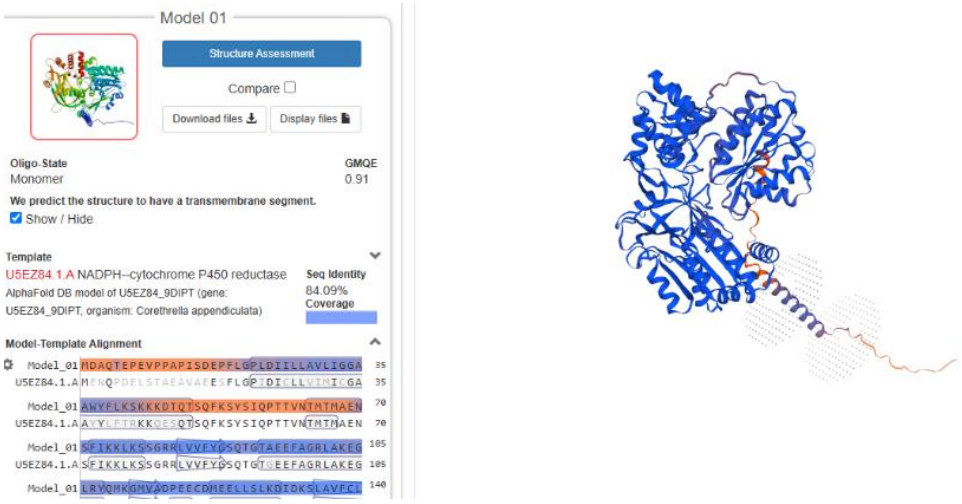
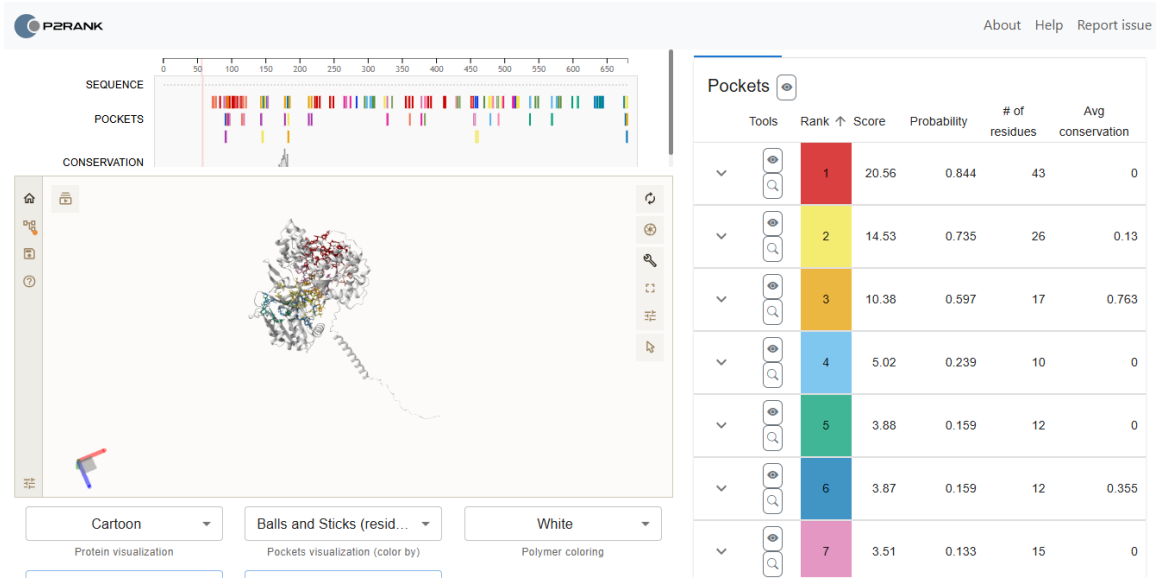


Fig 2: Swiss-Model output for Model 1 of *Cytochrome P450* from *Aedes aegypti*

The selected model exhibits mostly α-helices around a central pocket and was used for PrankWeb pocket prediction.

PrankWeb identified 14 total potential ligand-binding pockets. Among these, the top three pockets exhibited the highest scores and probabilities, pointing high confidence in their structural significance.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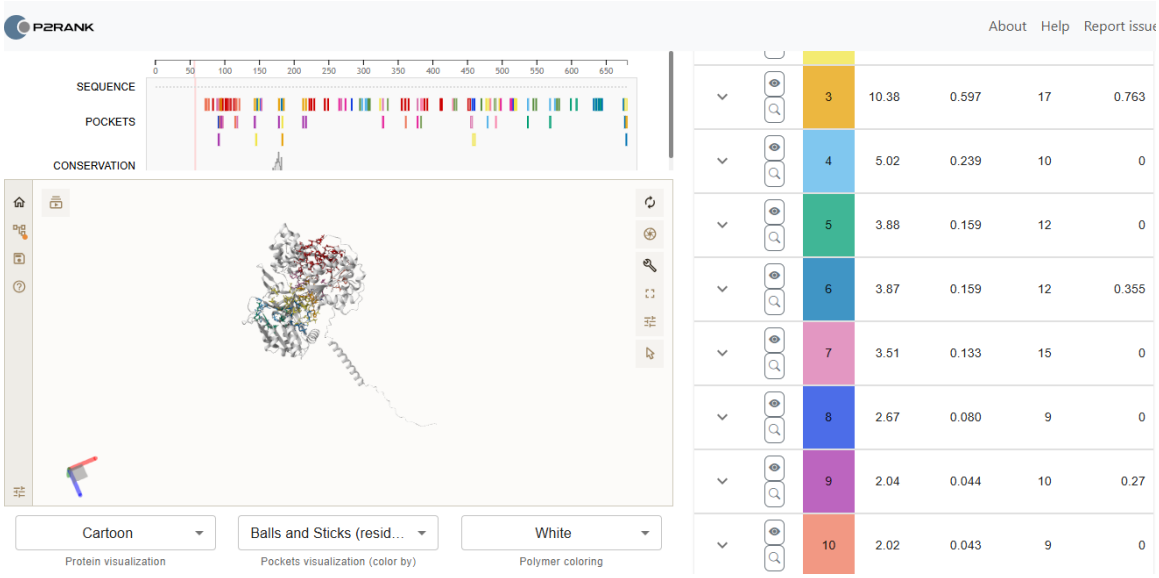
1 20.56 0.844 43 0
2 14.53 0.735 26 0.13
3 10.38 0.597 17 0.763
4 5.02 0.239 10 0
5 3.88 0.159 12 0
6 3.87 0.159 12 0.355
7 3.51 0.133 15 0

(a)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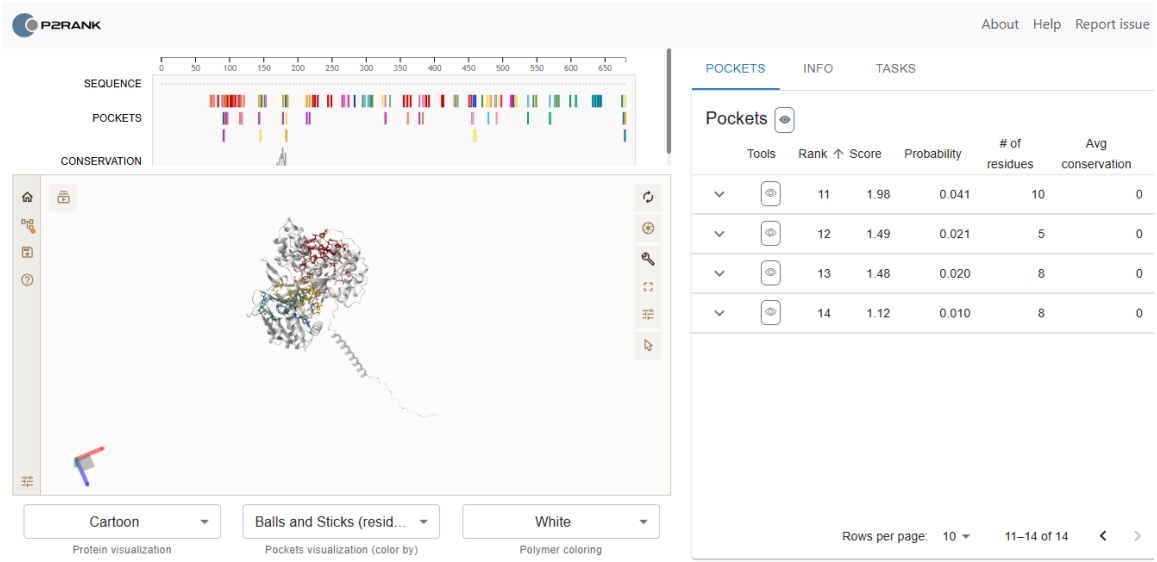
3 10.38 0.597 17 0.763
4 5.02 0.239 10 0
5 3.88 0.159 12 0
6 3.87 0.159 12 0.355
7 3.51 0.133 15 0
8 2.67 0.080 9 0
9 2.04 0.044 10 0.27
10 2.02 0.043 9 0

(b)



(c)

Fig 3: (a),(b),(c) Predicted binding pocket visualization of CYP450 from *Aedes aegypti* generated by PrankWeb

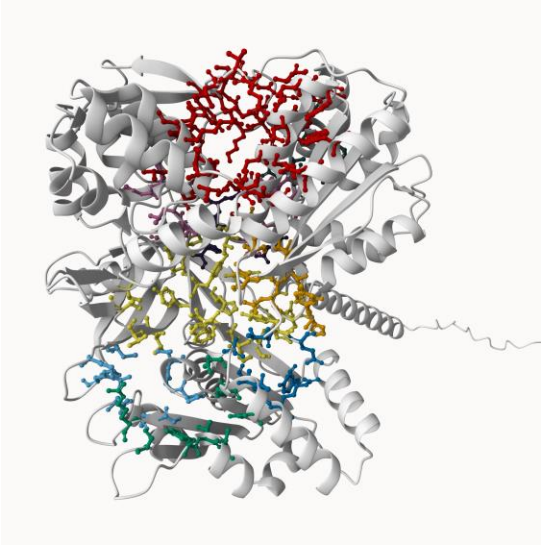


Fig 6: Close-up view of predicted pocket regions (colored regions) on the modeled 3D Structure

Pocket 1 (rank 1) highlighted in red consisted of 43 residues, which showed the highest score of 20.56 and probability of 0.844, indicating it is the large and most accessible pocket on the protein surface. However, its average conservation value

0.0 reveals low evolutionary conservation, suggesting that it may not be catalytically active but may act as a flexible binding region.

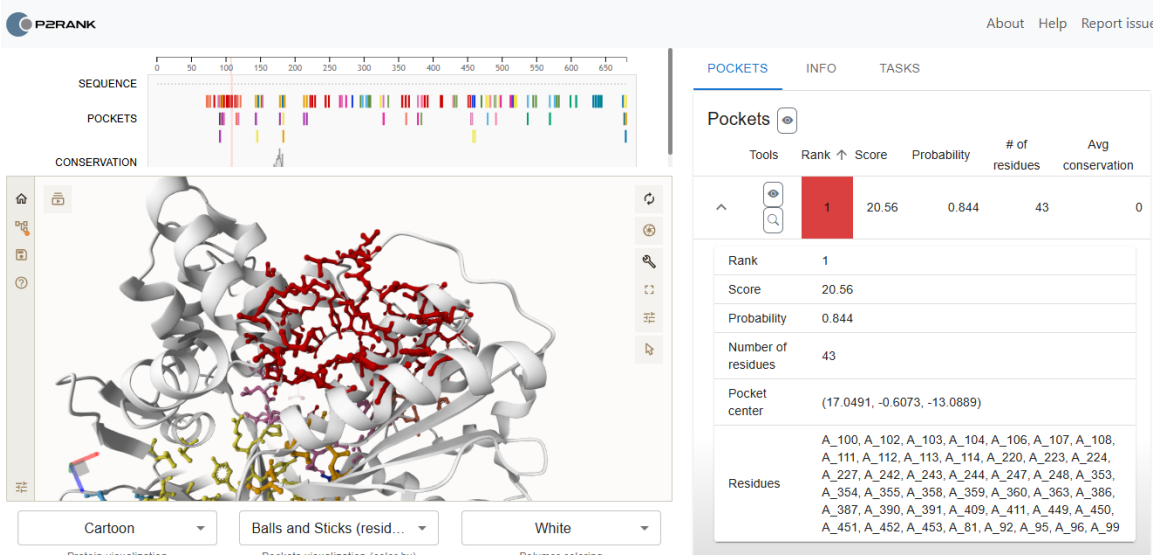


Fig 7: Binding Pocket 1 (Rank 1) highlighted in red showing the largest cavity (Score = 20.56, Probability = 0.844).

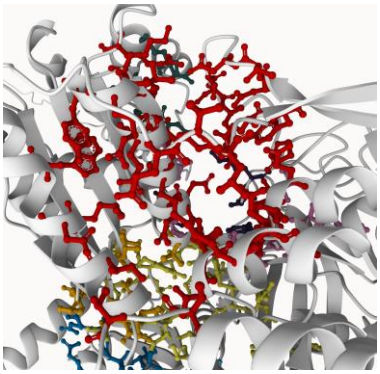


Fig 8: Close-up view of pocket 1 residues

Pocket 2 (rank 2) displayed a moderate score of 14.53 and probability of 0.735 with 26 residues and a slightly higher conservation score of 0.13. This pocket may serve as a secondary or regulatory binding site.

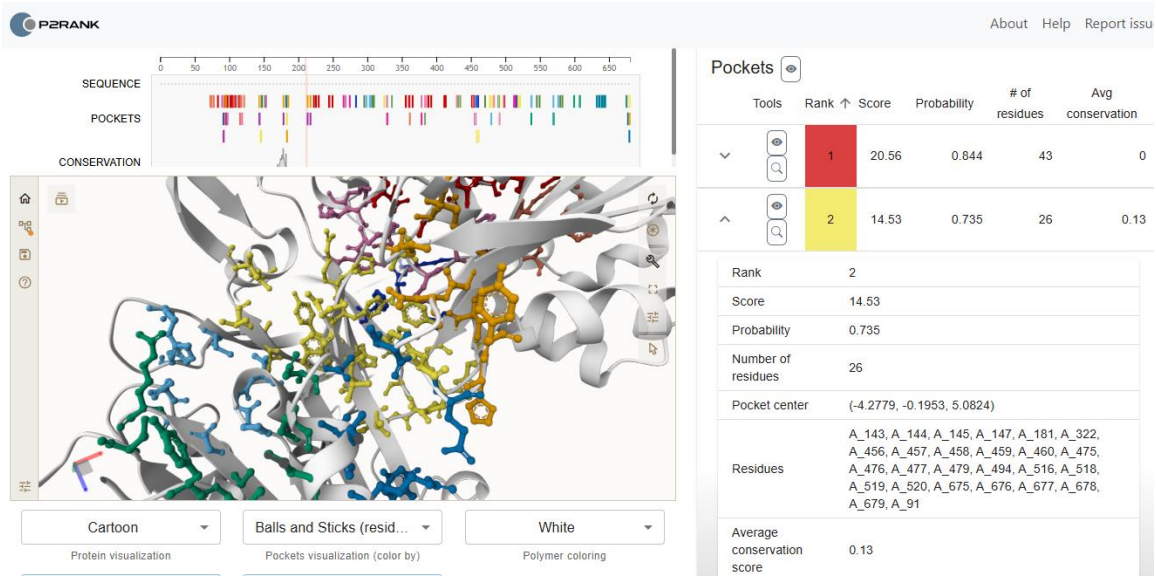


Fig 9: Binding Pocket 2 (Rank 2) with moderate cavity size (Score = 14.53, Probability = 0.735)

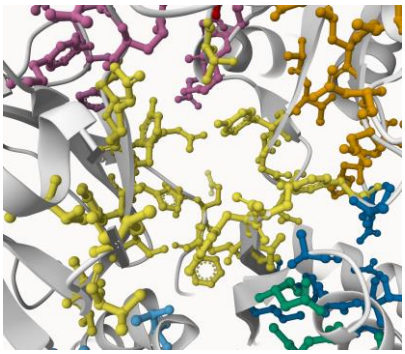


Fig 10: Zoomed-in view of Pocket 2 residues (Average Conservation = 0.13)

In contrast, Pocket 3 (rank 3) presented a relatively smaller cavity consisting of 17 residues with a moderate score of 10.38 and probability of 0.597. Notably, it exhibited the highest average conservation value of 0.763. Though pocket 3 may be smaller in size, it stands out with its high conservation which indicates that this site is evolutionarily preserved and probably plays a key role in catalysis and functioning of protein.

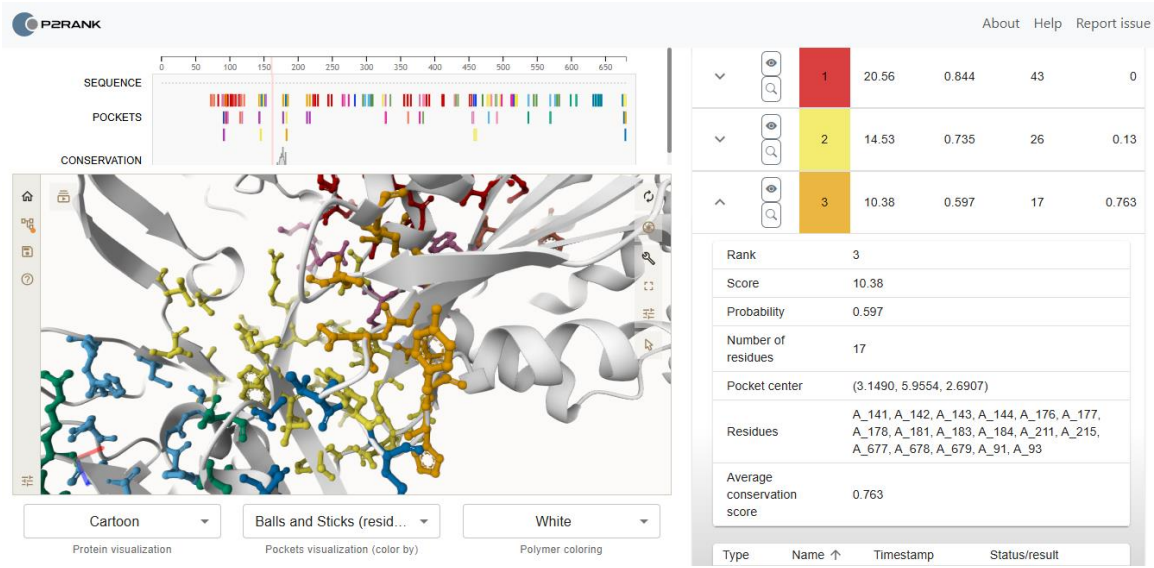


Fig 11: Binding Pocket 3 (Rank 3) with smaller but more conserved site (Score = 10.38, Probability = 0.597)

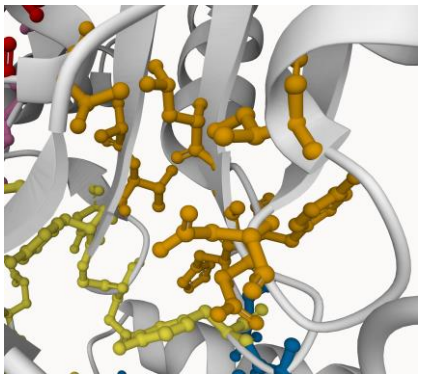


Fig 12: Enlarged view of Pocket 3 residues showing the most conserved catalytic region (Average Conservation = 0.763)

The remaining pockets (ranks 4–14) showed low probability values ranging from 0.239–0.010 and minimal conservation, suggesting they are likely non-functional surface cavity or structural voids. Overall, the results indicate that Pocket 1 stands out as a primary ligand-binding site based on geometric features, while Pocket 3 is the most probable candidate that may hold the key to the protein's catalytic function given its high evolutionary conservation across homologous proteins.

CONCLUSION

The predicted 3D model supports the specific structural features of Cytochrome P450, which are involved in xenobiotic metabolism, detoxification, and insecticide resistance mechanisms. This highlights its possible role in the resistance mechanisms of *Aedes aegypti*, the primary vector of dengue. These findings can lay out the groundwork for future lab experiments; drug design or studies targeting the function of this protein.

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