ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*

**Vol. 17, Issue, 01, pp.31405-31411, January, 2025**
**DOI: https://doi.org/10.24941/ijcr.48456.01.2025**

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

# RESEARCH ARTICLE

## A POTENTIAL TARGET OF MOSQUITO SPECIES USING MOLECULAR DOCKING

**Prabagaran, G., Dr. Sudarsanam, D., Sugapriya, P., Udayakumar, R., Balaji, R. and Dr. Praveena, P.**

Loyola College, Department of Advanced Zoology, School of Genomics.

**ARTICLE INFO**

***Article History:***
Received 20th October, 2024
Received in revised form
17th November, 2024
Accepted 24th December, 2024
Published online 31st January, 2025

***Key Words:***
CAVER, AUTODOCK.

****Corresponding author:***
**Prabagaran**

**ABSTRACT**

This field merges the biology and informatics, and one of the fastest growing areas, which is due to the development of computational tools and the databases. The area of pharmacology predicts the characteristics of a compound, its toxicity and interactions with a greater level of accuracy. This has a bigger advantage of making the researcher access the quality and the efficacy of type compounds before experimental work.



**Citation: Prabagaran, G., Dr. Sudarsanam, D., Sugapriya, P., Udayakumar, R., Balaji, R. and Dr. Praveena, P. 2024.** "A potential target of mosquito species using molecular docking *". International Journal of Current Research*, 17, (01), 31405-31411.

# INTRODUCTION

The mosquito's saliva is transferred to the host during the bite, and can cause an itchy rash. In addition, blood-feeding species can ingest pathogens while biting, and transmit them to other hosts. Those species include vectors of parasitic diseases such as malaria and filariasis, and arboviral diseases such as yellow fever and dengue fever. By transmitting diseases, mosquitoes cause the deaths of over 725,000 people each year. The vectors are the carrier of various diseases caused by pathogens, namely mosquitoes, flies, ticks, lice and bugs, not all the pathogens spread diseases to human, certain pathogens are aburden to plants and animals. Those diseases which are transmitted to humans by the mode of such pathogens are the vector borne diseases. These pathogens inspite of being tiny creatures, also are the origin of various diseases like dengue, chikungunya, malaria, yellow fever, filariasis and zika virus (Onen *et al.,* 2023).

# MATERIALS AND METHODS

Multiple sequence alignment of AchE from A.gambiae, A.albopictus and C.pipiens The stepwise procedure for performing the multiple sequence alignment is as follows
Example: >Anopheles_gambiae_AChE
MKTFFLLLIGTLAVG...
>Aedes_albopictus_AChE
MKTFLLLIGTLAAG...
>Culex_pipiens_AChE
MKTFFLLLIGTLAVG...

Replace the sequence fragments with actual AChE sequences from the respective species.

**Step 2:** Perform Multiple Sequence Alignment
ESPript itself is primarily a visualization tool rather than an alignment tool. For multiple sequence alignment, use other alignment tools like Clustal Omega, MAFFT, MUSCLE, or T-Coffee before visualizing the alignment using ESPript **(Gouet *et al.* 2003 and Sievers etal. 2018).**

**Using Clustal Omega**

- Go to the Clustal Omega web server.
- Upload your FASTA file containing the three AChE sequences.
- Perform the alignment.
- Download the resulting alignment in FASTA format.

Once the sequence alignment was performed using one of the above methods, the results were visualized in ESPript.

**Step 3:** Visualizing the Alignment with ESPript
ESPript generates publication-quality figures from aligned sequences. To visualize your

**AChE alignment:**

- Access ESPript:
- Go to the ESPript web server.
- Upload Alignment:
- Paste the FASTA-formatted multiple sequence alignment (from Clustal
- Omega,) into the text box provided on the ESPript web server.
- Configure Settings:
- Select visualization options (e.g., coloring schemes, formatting, conservation scores, numbering styles). You can choose options such as highlighting conserved residues, showing secondary structure information, or adjusting the display to emphasize sequence conservation.
- Generate Output:
- Click Submit or the corresponding button to generate the alignment
- visualization.

**Download the Output**

ESPript will generate the visualization as an image. PROTEIN MODELING USING ALPHAFOLD. Modeling proteins using AlphaFold 2.0, a deep learning-based tool developed by DeepMind, involves a complex series of steps that combine biological insights with advanced computational techniques (**Jumper *et al.* 2021 and Akdel** *et al*. 2021). Here is a detailed procedure for protein modeling using AlphaFold 2.0:

**Input Data Preparation**

The following steps are performed to generate the model:
Amino Acid Sequence: The primary input to AlphaFold 2.0 is the target protein's amino acid sequence. This sequence is a string of letters (from A to Z, representingthe 20 standard amino acids) representing the protein's primary structure. Here, the amino acid sequence corresponding to the crystal structure 6ary encoding for A. gambiae mutant AchE is provided. Template Structures (Optional): AlphaFold can use known 3D structures of homologous proteins (templates) for better prediction. These templates are retrieved from protein structure databases such as the Protein Data Bank (PDB) that shares maximum identity with the 6ary. Feature Extraction: AlphaFold 2.0 also requires the extraction of various additional features, such as: Pairwise distances between amino acids, Predicted secondary structure information, Solvent accessibility predictions, etc.

**Alpha Fold 's Deep Learning Model:** Alpha Fold 2.0 uses a deep learning model, specifically a transformer-based architecture, which consists of two main stages: Evoformer: This neural network module processes the multiple sequence alignments (MSA) and their associated features. The Evoformer utilizes a multi-layer attention mechanism to capture long-range dependencies in the protein sequence, effectively learning the evolutionary and structural information from the MSAs. Structure Module: The structure module converts the embeddings from Evoformer into 3D coordinates. This module predicts the 3D structure of the protein by iterating through various steps: Predicts distances between pairs of residues, Predicts the local geometry of each residue, including angles and torsions, Generates 3D coordinates for the entire protein chain. This module also refines the structure iteratively using the embeddings and evolutionary features generated by the Evoformer.

**Prediction of the Final Protein Structure:** The final predicted protein structure is generated in the following steps: Energy Minimization: After the initial structure is predicted, AlphaFold performs energy minimization to refine the geometry of the protein structure. This helps in optimizing bond angles, bond lengths, and the overall 3D fold. Confidence Score (pLDDT): AlphaFold generates a confidence score for the prediction, called the predicted Local Distance Difference Test (pLDDT). This score ranges from 0 to 100 and measures how confident the model is in the accuracy of its structure prediction. A score above 70 is generally considered high confidence, while scores below 50 indicate regions of lower confidence.

5. Post-processing the Prediction Once the model predicts the structure, some additional processing steps may be applied: Refinement and Validation: The predicted structure is further validated by checking the stereo chemical quality of the model (Pradeepkiran *et al*. 2021). This involves examining factors like: Ramachandran plot: Evaluates the distribution of dihedral angles of the amino acid residues. Clash scores: Ensures that atoms within the protein are appropriately spaced. Visualization: The final 3D structure can be visualized using molecular visualization software like Py MOL, Chimera, or VMD.

**ACTIVE SITE PREDICTION USING CAVER**

**Input Structure:** Active site prediction using CAVER is a computational approach used to identify and analyze tunnels and pockets in enzyme structures, which are crucial for substrate access and product release. This method is particularly useful in enzyme engineering and understanding enzyme-substrate interactions (Heinamann *et al*. 2021 and Vavra *et a*l. 2023). CAVER is a tool that helps predict and analyze tunnels and cavities in enzyme structures, which are essential for understanding how substrates access buried active sites. This is crucial for enzymes with complex structures where direct access to the active site is not apparent. CAVER requires a 3D protein structure, typically a PDB (Protein Data Bank) file. This structure can be experimentally determined via techniques like X-ray crystallography and NMR or generated computationally (e.g., via homology modeling). The crystal structure (PDB ID: 6ary) corresponding to A. gambiae AchE is given as the query input structure.

**Preprocessing and Surface Calculation:** The protein's 3D structure is (PDB ID: 6ary) corresponding to A. gambiae AchE processed to identify the solvent-accessible surface. This is crucial because many channels or cavities that could serve as active sites are usually exposed to the solvent or are cavities within the structure. The algorithm calculates the solvent-accessible surface area (SASA) and the surface pockets (Petreck *et al*. 2006). These pockets are potential binding sites or channels.

**Channel Detection:** The tool uses a geometrical approach to detect and visualize channels and cavities. It typically involves: Voronoi diagrams: Dividing the space into regions based on the positions of the protein atoms and identifying regions that may act as tunnels or channels. Grid-based analysis: CAVER often uses a 3D grid to analyze the proteinstructure and identify interconnected cavities that may form pathways.

**Pathway algorithm:** CAVER employs algorithms to find paths through the protein's interior based on accessible spaces. It typically uses methods like the Dijkstra algorithm or breadth-first search to compute the most probable paths from the protein's surface to internal cavities or channels. Pathway Characterization: Once potential channels are identified, CAVER characterizes them based on several factors:

**Size of the channel:** Identifying whether the pathway is large enough to allow the passage of molecules (e.g., small ions, water, or substrates). o Connectivity: Determining how connected the cavity is to other parts of the protein or whether it opens directly to the exterior. Functional relevance: Using knowledge of the protein (e.g., enzyme activity, ligand binding) to predict whether a particular cavity or channel is functionally important, such as being an active site for catalysis or a ligand-binding site.

**Surface and Interior Analysis:** CAVER doesn't just identify external channels but also investigates deeper, internal cavities that might serve as transport or catalytic pathways. These internal channels are often of interest in the context of enzymes or other functional proteins. The software can distinguish between buried channels (important for enzymatic action or molecular transport) and surface-exposed channels (important for ligand or substrate binding). 6. Visualization: CAVER provides a visualization of the predicted active sites or channels, typically in3D. This is done by rendering the protein structure and displaying the channels as tunnels or cavities with varying colors or transparency, making it easier to identify possible active sites visually. The visualization is often enhanced with a probe sphere method, where a sphere is moved through the identified channel to simulate the diffusion of molecules (like water or small ions) through the cavity.

**MOLECULAR DOCKING ANALYSIS:** Molecular docking is a computational technique used to predict the preferred orientation of asmall molecule (ligand) when bound to a target protein, which is crucial in drug discoveryand design. This method helps in understanding the interaction between the ligand and the. protein, estimating binding affinities, and facilitating virtual screening of potential drugcandidates (Pawar *et al.* 2023 and Chaudhary et al. 2024). All compounds attained from the PubChem database were employed for docking studies to confine the number of potentialhits from the virtual screening. Firstly, the open babel software 3-1-1 was used to convert the SDF format ofeach ligand to 3D PDB format (O'Boyle et al. 2011). AutoDock Vina was employed, the grid box was placed in the active site of the 6ary protein, and the docking process was initiated (Rauf et al. 2015). Lamarckian Genetic Algorithm was selected, and 10 poses were generated. During the docking, the protein structure was kept rigid while all the ligand's binding modes were maintained to be rotated and flexible during the binding. In the post-docking procedure, the output docking poses were analyzed to predict their binding affinity and the best poses were selected. The ligands with better docking scores (kcal/mol) values were ranked accordingly, and the STD docking score was chosen as a reference. A total of 21 ligands were found to have the best docking score and were ranked in the order. The compounds interaction with the AchE active site were assessed using theDiscovery Studio visualizer and Pymol (Lill *et al.* 2011 and Yuan *et al*. 2017). The docking and results interpretation are done using previously described methods.

# RESULTS AND DISCUSSION

Multiple Sequence Alignment (MSA) is a bioinformatics method for aligning three or more biological sequences (DNA, RNA, or protein sequences) to identify regions of similarity and infer functional, structural, or evolutionary relationships. MSA is fundamental for analysing homologous sequences, comparing genomes, and discovering evolutionary patterns. Importance of Multiple Sequence Alignment 1. Understanding Evolutionary Relationships:

MSA allows the comparison of sequences from different species or within gene families to identify conserved regions and infer phylogenetic relationships. Conserved sequences across species often indicate common ancestry and evolutionary conservation, while mutations provide insights into evolutionary divergence. 2. Identifying Conserved Functional Regions: Functional elements in biological sequences (such as active sites in proteins or regulatory motifs in DNA) are often conserved through evolution. MSA helps pinpoint these conserved regions, which can reveal their biological roles or importance in the molecule's structure and function. 3. Structural and Functional Prediction: Sequence alignment identifies patterns of conservation and variation that can help predict the structure or function of a gene or protein. Homologous sequences aligned using MSA can help model the three-dimensional structure of proteins by aligning them with sequences of known structure.

**Identifying Mutation Impact:** 9. Comparing homologous sequences allows researchers to assess the effects of mutations on function or structure. Mutations in conserved regions are more likely to be deleterious or disease-associated.

**Facilitating Drug Discovery:** In structural biology and drug discovery, MSA can highlight conserved binding sites or structural domains across homologous proteins, allowing the design of drugs targeting shared regions in related pathogens or disease mechanisms. 6. Genome Assembly and Annotation: MSA supports the assembly of sequenced DNA fragments by aligning them to reference genomes, helping resolve complex repeats or overlapping sequences. 7. Detecting Gene Duplication and Horizontal Gene Transfer: By comparing multiple sequences, MSA can reveal gene duplication events, gene losses, or horizontal gene transfer across different species. 8. Studying Population Genetics: MSA allows researchers to align individual sequences from populations to detect variations, such as SNPs (single nucleotide polymorphisms), which are important for understanding genetic diversity, evolutionary history, and disease susceptibility. Applications of MSA MSA is applied across various fields of bioinformatics and molecular biology (Ibrahim et al. 2024 and Ishaeq et al. 2019) Phylogenetics and

Evolutionary Biology: Understanding evolutionary relationships and constructing phylogenetic trees. Structural Biology: Predicting structural features and 3D structure of proteins by aligning homologous sequences. Strengthening genetic surveillance to monitor resistance mutations in mosquito.

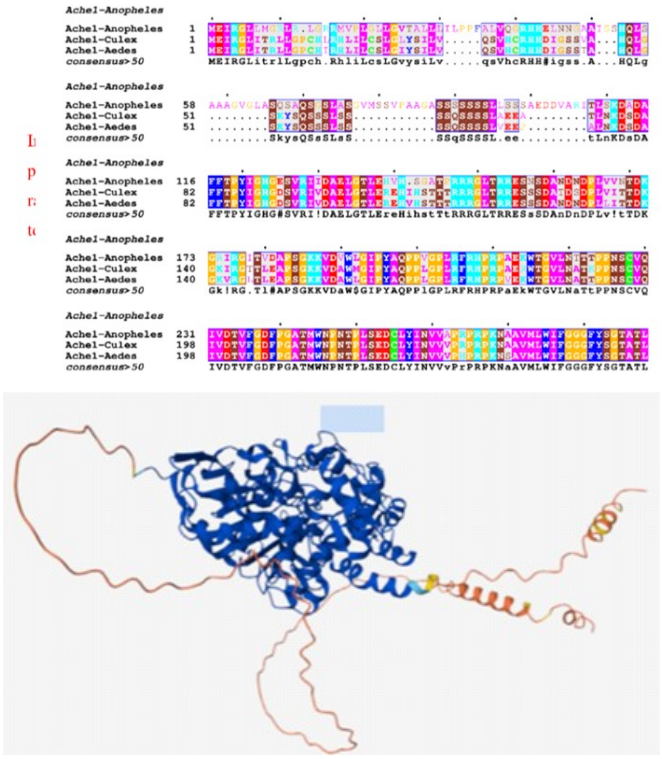
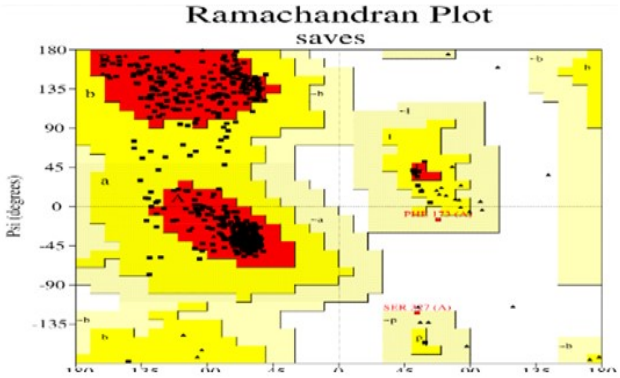


**Figure 2. Alphafold-based model of A. albopictus**




**STRUCTURE VALIDATION USING G-FACTOR:** The G-factors mentioned in the document are statistical measures used to assess the quality of a protein structure, particularly in the context of its conformational stability. They provideinsights into the geometry of the protein's backbone and side chains (Yasuda *et al.* 2022). Here's a breakdown of their significance: 1. G-factors Overview: G-factors are calculated for dihedral angles (phi, psi, chi1, chi2, etc.) and other structural parameters. They help evaluate how well the proteinstructure adheres to expected geometric norms. 2. Interpretation of Values: Positive G-factors: Indicate that the conformation is more favorable than average, suggesting that the residues are stable and energetically favorable. o Negative G-factors: Suggest that the conformation is less favorable, indicating potential steric clashes or unfavorable interactions within theprotein structure.

**Overall Assessment:** The overall G-factor summarizes the protein's structural quality. A higher overall G-factor indicates a more stable and well-formed structure, while alower value may suggest issues that could affect the protein's function or stability. 4. Use in Validation: G-factors are often used in conjunction with other validation tools (like Ramachandran plots) to assess the reliability of protein models, especially thosegenerated through computational methods or homology modelling Based on the analysis, we found that out of 10, 7 g-factors are positive, and only threeg-factors are negative, which indicates the good quality of the modeled protein structure. C. pipiens modeled structure The Alpha Fold-generated model of the Acetylcholinesterase protein (UniProt ID: Q86G68) provides a predicted three-dimensional structure based on the protein's amino acid sequence. Model Confidence: The model includes a confidence score known as pLDDT (predicted. Local Distance Difference Test), which ranges from 0 to 100. A higher score indicates greater confidence in the predicted structure. For example, regions with a pLDDT greater than 90 are considered to have very high confidence, while scores between 70 and 90 indicate moderate confidence. Regions with lower scores may be less reliable and unstructured in isolation.

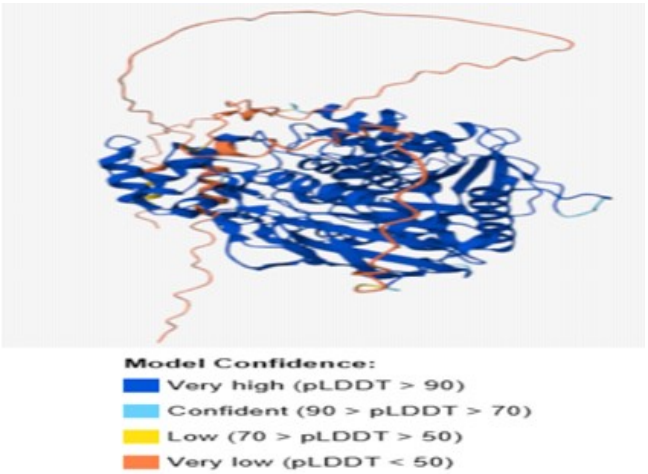



**Figure 4. Alphafold-based model of C. pipiens**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Y282 | V235 | S280 | D233 | V232 | Y282 | Q198 | C228 |
| S283 | W245 | S283 | T234 | D233 | S283 | P199 | V229 |
| Y291 | N246 | Y291 | F236 | A242 | Y291 | P200 | Q230 |
| E359 | S280 | E359 | D238 | W245 | E359 | A212 | I231 |
| S360 | Y291 | S360 | F239 | N246 | S360 | C228 | V232 |
| W441 | E359 | Y489 | A242 | P247 | A361 | V230 | D233 |
| F449 | S360 | F490 | T243 | S280 | W393 | Q230 | A242 |
| Y489 | F449 | S587 | W245 | Y282 | I446 | I231 | W245 |
| F490 | Y489 | N590 | N246 | S283 | C447 | V232 | N246 |
| Y493 | F490 | P591 | P247 | L288 | F449 | D233 | P247 |
| H600 | Y493 | W592 | S280 | Y291 | Y489 | A242 | S280 |
| I604 | H600 | M599 | Y282 | E359 | F490 | W245 | Y282 |
| G278 | I604 | H600 | S283 | S360 | H559 | N246 | S283 |

**Frequent Codes:** The code W245 appears most frequently across multiple tunnels, indicating it may be a significant marker or trait in the study context. In addition, W245 which is found in the active site has the ability to interact with the inhibitor. Other frequently occurring codes include Y291, S280, and D233, which also appear in several tunnels.

| Name | S. No | Tunnel 1 | Tunnel 2 | Tunnel 3 | Tunnel 4 |
|---|---|---|---|---|---|
| | | | | | |
| A.albopictus | | P166 | P162 | C195 | C195 |
| | | P167 | A164 | V196 | Q197 |
| | | L168 | Q165 | Q197 | I198 |
| | | R172 | P166 | I198 | V199 |
| | | C195 | P167 | V199 | D200 |
| | | V196 | V179 | D200 | M211 |
| | | Q197 | C195 | N213 | Q212 |
| | | E220 | V196 | P214 | N213 |
| | | C222 | C195 | Y249 | P214 |
| | | L223 | V196 | S250 | Y249 |

**Potential Implications:** The presence of certain codes in multiple tunnels could suggest common traits among populations, while unique codes in specific tunnels might indicate localized adaptations or variations. Current antimalarial drug development research has two main objectives: (1) finding candidates that can target resistant strains and finding extremely potent prospects that can be given in shorter treatment durations (Belete *et al.* 2020 and Kumar *et al.* 2020). Using partner medications, in which artemisinin or a derivative is combined with another medication to provide the desired therapeutic effect, is one of the remedies found for this problem. Still, more study is required to determine whether the treatment may be shortened or reduced to a single dose. Furthermore, as hypnozoites of P. vivax and P. ovale can cause several malaria episodes in a single infection, candidates that may target both the asexual and sexual stages and these parasites are exciting (Noviyanti *et al.* 2022). Recently, artemisinin-hybridized compounds) have been tested against malaria to address artemisinin resistance. Dihydroartemisinyl-chalcone esters, for instance, are effective against strains resistant to and Temperature facilities in tropical regions where malaria is frequently endemic. Another groupOf intriguing antimalarial candidates includes hybrids of artemisinin and other natural products, such as homoegonol and thymoquinone, which have high antiplasmodial efficacy and potency, demonstrating better activity than chloroquine (Quadros *et al.* 2021).

| S.no | Compound Name | *A.gambiae* (kcal/mol) | *A.albopictus* (kcal/mol) | *C.pipiens* (kcal/mol) |
|---|---|---|---|---|
| 1. | 2,2-difluoro-1-(1-(pentan-3-yl)-1H-pyrazol-3-yl) ethan-1-one | -5.8 | -5.7 | -4.3 |
| 2. | Artemisinin (68827) | -8.4 | -7.2 | -6.0 |
| 3. | Anhydrodehydroartemisinin 6477581 | -8.6 | -7.7 | -6.0 |
| 4. | 9-Desmethylene 9-Oxo-artemisitene 21635426 | -7.8 | -5.3 | -5.0 |
| 5. | N-Hydroxy-11-azaartemisinin 25169950 | -7.7 | -7.4 | -5.4 |

**Aminoquinol:** Docking Score: -9.6 kcal/mol This compound also exhibits a very strong binding affinity, indicating it could be another promising candidate for targeting A. gambiae. A. albopictus 1. Cotecxin: Docking Score: -8.6 kcal/mol Cotecxin demonstrates the best binding affinity for A. albopictus, suggesting it may be particularly effective against this species.

**Hydroxychloroquine Sulfate:** Docking Score: -8.6 kcal/mol This compound ties with Cotecxin for the best score, indicating it also has a strong potential for interaction with the target proteins in A. albopictus.

**LIGAND-PROTEIN INTERACTION OF DOCKED COMPLEXES:** A. gambiae the crystal structure of A.gambiae AchE (PDB ID: 36ary) and modelled Ach Efro A.albopictus and C.pipiens was employed for docking studies. 2,2-difluoro-1-(1-(pentane-3-yl)-1H-pyrazol-3-yl) ethan-1-one, when docked with mutant AchE, showed it has similar interaction, which was observed in the crystal structure. For instance, Tyr489, Tyr291, Tyr245 and Gly278 formed hydrogen bonds with the protein. Followingly, with the 3-methychloroquine, Tyr291 and Tyr489 form the hydrogen bond interaction. Similar hydrogen bond interaction with Tyr291 was found in aminoquinol docked A.gambiae AchE protein. Based on the docking score, it is evident that these two compounds, 3-methychloroquine and aminoquinol could be potentially used as drug molecule for resistant GMO A.gambiae.

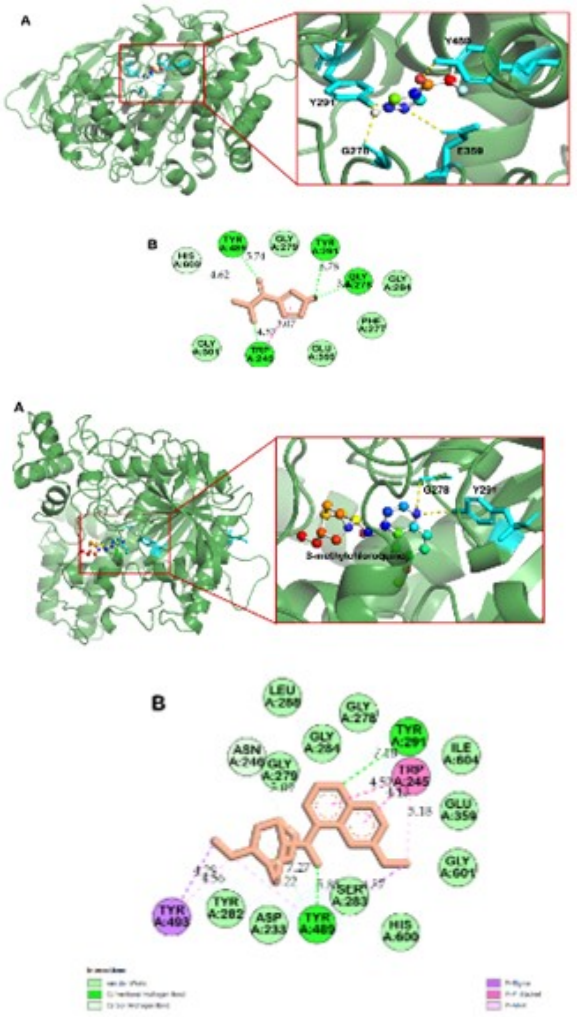



**Figure 8. 3D (A) and 2D (B) interaction of 3-methylchloroquine with mutant AchE of A. Gambiae**

The docking studies of new compounds against mutant acetylcholinesterase (AchE) have shown promising results in the search for effective antimalarial treatments, particularly in the context of increasing resistance to traditional therapies like artemisinin. This discussion will explore the significance of these findings, comparing the efficacy of novel compounds with that of artemisinin. Acetylcholinesterase is an enzyme that plays a crucial role in the nervous system of insects, including malaria vectors such as Anopheles gambiae. Mutations in the AchE gene can leadto resistance against certain insecticides, complicating malaria control efforts.

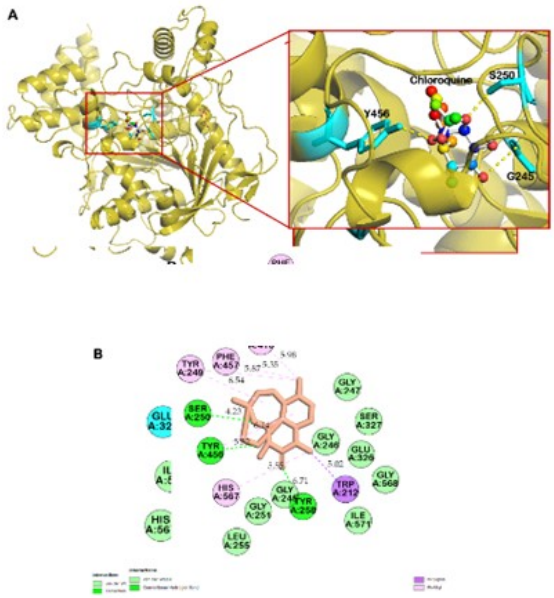



**FIGURE 10.** 3D (A) and 2D (B) interaction of 2,2-difluoro-1-(1-(pentane-3-yl)-1H-pyrazol-3-yl) ethan-1-one docked with mutant AchE of A. albopictus

The emergence of resistance to artemisinin, the cornerstone of malaria treatment, has prompted researchers to explore alternative compounds that can effectively target resistant strains of the malaria parasite and its vectors. Molecular docking is a computational technique that predicts the preferred orientation of a ligand (in this case, a potential drug) when bound to a target protein (like mutant AchE). This method allows researchers to evaluate the binding affinity and interaction patterns of various compounds, providing insights into their potential efficacy as therapeutic agents. New Compounds vs. Artemisinin Efficacy Against Mutant AchE: Recent studies have identified new compounds, such as 2,2-difluoro-1-(1- (pentane-3-yl)-1H-pyrazol-3-yl) ethan-1-one, which have shown significant binding interactions with mutant AchE. These compounds demonstrated similar or even superior docking scores compared to artemisinin derivatives, indicating a strong potential for inhibiting the enzyme's activity , . In contrast, artemisinin primarily targets the malaria parasite itself rather than the vector's AchE. While artemisinin is effective against the parasite, its efficacy is compromised by the development of resistance, necessitating the exploration of alternative compounds that can target both the parasite and its vectors.

The new compounds often exhibit unique mechanisms of action that differ from those of artemisinin. For instance, they may form specific hydrogen bonds with key residues in the AchE active site, disrupting its function more Effectively than artemisinin, which relies on reactive oxygen species to exert its effects on the parasite , The ability of these new compounds to interact with the AchE of resistantstrains suggests that they could be used in combination therapies, potentially enhancing the overall efficacy of malaria treatment regimens. The rational design of new compounds based on structural information from docking studies has opened avenues for developing drugs with improved pharmacokinetic properties and safety profiles. This is particularly important in light of the challenges posed by artemisinin resistance and the need for novel therapeutic strategies .The identification of compounds that can effectively inhibit mutant AchE could lead to the development of new insecticides or combination therapies that enhance the effectiveness of existing antimalarial drugs.

The exploration of new compounds through molecular docking against mutant acetylcholinesterase represents a significant advancement in the fight against malaria, particularly in the context of rising resistance to artemisinin. By targeting the AchE of resistant strains, these compounds offer a promising alternative or complement to traditional therapies. Continued research in this area is essential to develop effective malaria control and treatment strategies, ensuring that we can combat both the parasite and its vectors effectively. The integration of computational techniques like molecular docking into drug discovery processes will be crucial in identifying and optimizing new therapeutic candidates. From the attained results, this study compares docking scores for three mosquito species: A. gambiae, A. albopictus, and C. pipiens. A. gambiae exhibited the broadest range of docking scores (-9.6 to -5.8 kcal/mol), indicating significant variability in binding affinities, suggesting a diverse interaction profile with the tested compounds. A. albopictus showed a narrower range (-8.2 to -5.3 kcal/mol), while C. pipiens had the narrowest range (-6.2 to -4.3 kcal/mol), indicating less variability in binding affinities. The strongest binding affinities for A. gambiae were observed with 3-Methylchloroquine (-9.5 kcal/mol) and Aminoquinol (-9.6 kcal/mol), highlighting their potential as effective candidates for targeting this species. Additionally, structural validation using the Ramachandran plot confirmed the reliability of the modelled structures for further studies, and CAVER analysis revealed that A. gambiae had the highest number of tunnels (8), potentially linked to a mutation in the AchE protein, which may influence its biological activity.

# REFERENCES

Gouet, P., Robert, X., & Courcelle, E. (2003). ESPript/ENDscript: extracting and renderingsequence and 3D information from atomic structures of proteins. Nucleic acids research, 3113, 3320-3 . https://doi.org/10.1093/nar/gkg556.

Sievers, F., & Higgins, D. (2018). Clustal Omega for making accurate alignments of many protein sequences. Protein Science, 27. https:// doi.org/ 10.1002/ pro.3290.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S., Ballard, A., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., , A., Kavukcuoglu, K., Kohli, P., & Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. Nature, 596, 583 - 589. https://doi.org/10.1038/s41586-021-03819-2.

Akdel, M., Pires, D., Pardo, E., Jänes, J., Zalevsky, A., Mészáros, B., Bryant, P., Good, L., Laskowski, R., Pozzati, G., Shenoy, A., Zhu, W., Kundrotas, P., Serra, V., Rodrigues, C., Dunham, A., Burke, D., Borkakoti, N., Velankar, S., Frost, A., Lindorff-Larsen, K., Valencia, A., Ovchinnikov, S., Durairaj, J., Ascher, D., Thornton, J., Davey, N., Stein, A., Elofsson, A., Croll, T., & Beltrão, P. (2021). A structural biology community assessment of AlphaFold2 applications. Nature Structural & Molecular Biology, 29, 1056 - 1067. https://doi.org/10.1038/s41594-022-00849-w.

Heinemann, P., Armbruster, D., & Hauer, B. (2021). Active-site loop variations adjust activity and selectivity of the cumene dioxygenase. Nature Communications, 12. https://doi.org/10.1038/s41467-021-21328-8.

Vávra, O., Tyzack, J., Haddadi, F., Stourac, J., Damborský, J., Mazurenko, S., Thornton, J., & Bednář, D. (2023). Large-scale annotation of biochemically relevant pockets and tunnels in cognate enz yme–ligand complexes. Journal of Cheminformatics, 16. https://doi.org/10.1186/s13321-024-00907-z.

Pawar, S., Kulkarni, C., Gadade, P., Pujari, S., Kakade, S., Rohane, S., & Redasani, V. (2023). Molecular Docking using different Tools. Asian Journal of Pharmaceutical Research. https://doi.org/10.52711/2231-5691.2023.00053.

Chaudhary, M., & Tyagi, K. (2024). A REVIEW ON MOLECULAR DOCKING AND ITS APPLICATION. International Journal of Advanced Research. https://doi.org/10.21474/ijar01/18505.

Pradeepkiran, J., Sainath, S., Balne, P., & Bhaskar, M. (2021). Computational modeling and evaluation of best potential drug targets through comparative modeling. , 39-78. https://doi.org/10.1016/B978-0-323-85681-2.00001-X.

Petrek, M., Otyepka, M., Banáš, P., Kosinová, P., Koča, J., & Damborský, J. (2006). CAVER: a new tool to explore routes from protein clefts, pockets and cavities. BMC Bioinformatics, 7, 316 - 316. https://doi.org/10.1186/1471-2105-7-316.

O'Boyle, N., Banck, M., James, C., Morley, C., Vandermeersch, T., & Hutchison, G. (2011). Open Babel: An open chemical toolbox. Journal of Cheminformatics, 3, 33 - 33. https://doi.org/10.1186/1758-2946-3-33.

Rauf, M., Zubair, S., & Azhar, A. (2015). Ligand docking and binding site analysis with pymol and autodock/vina. International Journal of Basic and Applied Sciences, 4, 168-177. https://doi.org/10.14419/IJBAS.V4I2.4123.

Lill, M., & Danielson, M. (2011). Computer-aided drug design platform using PyMOL. Journal of Computer-Aided Molecular Design, 25, 13-19. https://doi.org/10.1007/s10822-010-9395-8.

Yuan, S., Chan, H., & Hu, Z. (2017). Using PyMOL as a platform for computational drug design. Wiley Interdisciplinary Reviews: Computational Molecular Science, 7. https://doi.org/10.1002/wcms.1298.

Ibrahim, M., Yusof, U., Eisa, T., & Nasser, M. (2024). Bioinspired Algorithms for Multiple Sequence Alignment: A Systematic Review and Roadmap. Applied Sciences. https://doi.org/10.3390/app14062433.

Ishaq, M., Khan, A., Khan, M., & Imran, M. (2019). Current Trends and Ongoing Progress in the Computational Alignment of Biological Sequences. IEEE Access, 7, 68380-68391. https://doi.org/10.1109/access.2019.2916154.

Adolfi, A., Poulton, B., Anthousi, A., Macilwee, S., Ranson, H., & Lycett, G. (2019). Functional genetic validation of key genes conferring insecticide resistance in the major African malaria vector, Anopheles gambiae. Proceedings of the National Academy of Sciences of the United States of America, 116, 25764 - 25772. https://doi.org/10.1073/pnas.1914633116.

Wipf, N., Duchemin, W., Kouadio, F., Fodjo, B., Sadia, C., Mouhamadou, C., Vavassori, L., Mäser, P., Mavridis, K., Vontas, J., & Müller, P. (2022). Multi-insecticide resistant malaria vectors in the field remain susceptible to malathion, despite the presence of Ace1 point mutations. PLoS Genetics, 18. https://doi.org/10.1371/journal.pgen.1009963.

Njoroge, H., Hof, A., Oruni, A., Pipini, D., Nagi, S., Lynd, A., Lucas, E., Tomlinson, S., Grau-Bové, X., McDermott, D., Wat'senga, F., Manzambi, E., Agossa, F., Mokuba, A., Irish, S., Kabula, B., Mbogo, C., Bargul, J., Paine, M., Weetman, D., & Donnelly, M. (2021). Identification of a rapidly-spreading triple mutant for high-level metabolic insecticide resistance in Anopheles gambiae provides a real-time molecular diagnostic for antimalarial intervention deployment. Molecular Ecology, 31, 4307 - 4318. https://doi.org/10.1111/mec.16591.

Djogbénou, L., Labbé, P., Chandre, F., Pasteur, N., & Weill, M. (2009). Ace-1 duplication in Anopheles gambiae: a challenge for malaria control. Malaria Journal, 8, 70 - 70. https://doi.org/10.1186/1475-2875-8-70.

Cheung, J., Mahmood, A., Kalathur, R., Liu, L., & Carlier, P. (2018). Structure of the G119S Mutant Acetylcholinesterase of the Malaria Vector Anopheles gambiae Reveals Basis of Insecticide Resistance.. Structure, 26 1, 130-136.e2 . https://doi.org/10.1016/j.str.2017.11.021.

Engdahl, C., Knutsson, S., Fredriksson, S., Linusson, A., Bucht, G., & Ekström, F. (2015). Acetylcholinesterases from the Disease Vectors Aedes aegypti nd Anopheles gambiae: Functional Characterization and Comparisons with Vertebrate Orthologues. PLoS ONE, 10. https://doi.org/10.1371/journal.pone.0138598.

*******