**Highlights in BioScience**
**ISSN:2682-4043**
DOI:10.36462/H.BioSci.202501

**Research Article**

**Open Access**

[1] Biology Department, Faculty of Mathematics and Natural Sciences, University of Jember, Jember, East Java, Indonesia.

*** To whom correspondence should be addressed: senjarini@unej.ac.id**

**Editor:** Hatem Zayed, *Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, Qatar.*

**Reviewer(s):**
Laila Dabab Nahas, *Usher Institute, University of Edinburgh, Scotland, United Kingdom.*

Hala Abdelgaid, *National Hepatology and Tropical Medicine Research Institute (NHTMRI) Corniche El Nil, Imbaba, Giza 12651, Egypt.*

**Received:** December 20, 2024
**Accepted:** April 25, 2025
**Published:** May 5, 2025

**Citation:** Oktarianti R, Nurdianti F, Wathon S, Senjarini K. *In silico* study of the interaction between serotonin and D7 protein from the salivary gland of *Aedes aegypti*. 2025 May 5;8:bs202501



**Data Availability Statement**: All relevant data are within the paper and supplementary materials.
**Funding:** This research is supported by the National Research and Innovation Agency (BRIIN) through the Research and Innovation Program for Advanced Indonesia, Batch 3, under reference number 12/II.7/HK/2023
**Competing interests:** The authors declare that they have no competing interests.

# *In silico* study of the interaction between serotonin and D7 protein from the salivary gland of *Aedes aegypti*

Rike Oktarianti[1], Faranisa Nurdianti[1], Syubbanul Wathon[1], Kartika Senjarini[1]

## Abstract

Protein components of the salivary glands of disease vectors have been known to facilitate the blood-feeding process in the host body. The main component of the salivary glands of *Aedes aegypti* is the immunogenic D7 protein. During the blood-feeding process, the D7 protein can bind to biogenic amine compounds, such as serotonin, which is a neurotransmitter involved in platelet activation. This ability indicates that the D7 protein can inhibit the platelet aggregation process. This study aims to explore *in silico* the interaction between serotonin and the D7 protein from the salivary glands of *Ae. aegypti* using a molecular docking approach. The methods used in this study include the selection of the 3D structure of the D7 protein and serotonin ligand, preparation of the 3D structure of the D7 protein, native ligands, and test ligands, validation of the molecular docking method, and analysis and visualization of the molecular docking results. The results of molecular docking between the D7 protein and the serotonin ligand showed a $\Delta G$ value for the interaction of −9.25 kcal/mol. The serotonin ligand binds to the active site of the D7 protein through several amino acid residues, including GLU 158, ILE 175, ARG 176, TYR 178, TYR 248, ASP 265, and GLU 268. These amino acid residues of the D7 protein bind to atoms on the serotonin ligand through conventional hydrogen bonds, carbon hydrogen bonds, $\pi$-$\sigma$ bonds, $\pi$-$\pi$ T-shaped bonds, and $\pi$-alkyl bonds. Based on the *in silico* data, it is shown that the D7 protein from the salivary glands of *Ae. aegypti* can bind stably and spontaneously to serotonin ligands. This indicates that the D7 protein has potential as a platelet aggregation inhibitor agent for the development of drug discovery in the fields of health and pharmacy.

**Keywords:** *Aedes aegypti*, D7 protein, molecular docking, serotonin, platelet aggregation

## Introduction

The salivary glands of disease vector arthropods are known to be important organs for the success of the blood-feeding process in the host body [1]. This is because the salivary glands contain various bioactive components that can suppress the host's immune response. In general, components in the salivary glands of disease vectors act as vasodilator and immunomodulatory factors that can affect the host's hemostasis [2]. Several previous studies have identified components of the salivary glands of disease vectors that affect the host's immune response, including bioactive components in the salivary glands of *Aedes aegypti* as a vector of dengue fever [3; 4].

The salivary glands of *Ae. aegypti* contain various types of bioactive components in the form of protein molecules. The protein components of the salivary glands of *Ae. aegypti* generally include apyrase, aegyptin, serpin, and the D7 Family [5]. The apyrase protein has the activity to hydrolyze ATP into ADP and AMP, which can inhibit platelet activation [6]. Aegyptin is an allergen that can bind to collagen and von Willebrand factor, reducing the formation of blood clots [7]. Serpin is a protease inhibitor that inhibits the activity of serine proteases in various host hemostasis reactions [8]. The D7 protein is known to be the most abundant component in the salivary glands of *Ae. aegypti* [9]. *Ae. aegypti* performs the blood-feeding process on the host's body by inserting its proboscis into the skin layer until it reaches the endothelium of the blood vessels. The host's body responds to this action by releasing biogenic amine compounds to stop the blood flow through the mechanism of

platelet aggregation. During the blood-feeding process, *Ae. aegypti* releases the D7 protein component from its salivary glands into the host's body. The D7 protein has a high affinity for biogenic amine compounds, such as norepinephrine, histamine, and serotonin [10]. Serotonin is a type of biogenic amine that plays an important role in platelet activation [11]. When the D7 protein from the salivary glands of *Ae. aegypti* binds to serotonin, the process of platelet aggregation around the host's wound is inhibited, allowing the blood-feeding process to proceed smoothly. This activity indicates that the D7 protein can inhibit the platelet aggregation process in the host's body. D7 has the ability to block platelet aggregation, which makes it an excellent candidate for an anti-platelet medication. However, its effects on biogenic amines, including its interaction with serotonin from the salivary glands of *Ae. aegypti*, are not well characterized. One branch of biochemistry involves the study of proteinligand interactions, which can be explored through *in silico* approaches that predict molecular interactions using computer simulations. For instance, molecular docking analysis enables the identification of specific binding sites on target proteins for a test ligand [12].

Previous studies have primarily focused on the interaction between the D7 protein and leukotriene A4, demonstrating the formation of a stable and natural complex [13]. However, no prior research has specifically examined the ability of D7 from *Ae. aegypti* to bind serotonin, a crucial biogenic amine involved in platelet aggregation. This study addresses this gap by utilizing *in silico* molecular docking to explore the potential interaction between D7 and serotonin. By doing so, we aim to provide new insights into the functional role of D7 and its potential as a foundation for developing novel anti-platelet aggregation agents.

## Materials and Methods

### Downloading 3D structure D7 protein and ligand

The amino acid sequence of the D7 protein from *Ae. aegypti* was downloaded from the UniProt database with accession code P18153 [14]. The three-dimensional (3D) structure of the D7 protein was obtained from the SWISS-MODEL database [15]. The resulting model was selected based on its best quality and downloaded in `.pdb` file format [16]. The 3D model structure of the D7 protein uses the D7 protein with the template PDB id `3dye.1`. The model structure from this template includes a native ligand, which is *l*-norepinephrine. In this study, a test ligand was used to observe its interaction with the D7 protein. The test ligand selected was serotonin. The three-dimensional structure of serotonin was obtained from the PubChem database with accession code 5202. This serotonin entry originates from human metabolism. The serotonin molecule was downloaded from the PubChem database in `.sdf` file format [17].

### Preparation and optimization of the 3D structure of D7 protein and the native ligand *l*-norepinephrine

The preparation of the 3D structure of the D7 protein involves removing the native ligand, non-functional ligands, and water molecules. This preparation is carried out using AutoDock Tools software [18]. The first step in preparing the D7 protein structure is the removal of all water molecules and non-functional ligands. The D7 protein is then separated from its native ligand, *l*-norepinephrine. The structure of the D7 protein is saved in `.pdb` format, and the structure of *l*-norepinephrine is also saved in `.pdb` format within the same folder. The structure of the D7 protein is then optimized by adding polar hydrogen atoms and checking for missing atoms to ensure the integrity of the downloaded 3D structure. The D7 protein is subsequently charged using Kollman charges, resulting in charge neutralization. The optimized structure is saved in `.pdbqt` format [19].

Further optimization is performed on the 3D structure of the *l*-norepinephrine ligand. The ligand is optimized by adding Gasteiger charges, followed by a non-polar merge, with the expectation that only hydrogen atoms will be available to form bonds with the target protein residues (D7 protein) [20]. The native ligand is then prepared for docking by selecting rotation points using the torsion tree menu. This process identifies and assigns torsion points, improving the accuracy of ligand positioning predictions. The final optimized structure of the native ligand is saved in `.pdbqt` format in the same folder as the optimized D7 protein structure [19]. Preparation and optimization of the native ligands 3D structure were also performed using AutoDock tools software.

### Validation of the molecular docking method

The next step in the molecular docking phase is to validate the method through a re-docking process of the D7 protein with its native ligand, *l*-norepinephrine, which has already been prepared and optimized. The initial step of the re-docking process involves determining the interaction site by defining the grid area using a grid box. This stage is conducted using AutoDock tools software [18].

The grid box settings include the number of points in each dimension (x, y, and z), the spacing in Å, and the center coordinates of the grid box (x, y, and z). These settings are saved in the `.gpf` (grid parameter file) format. The validation of the molecular docking method between the D7 protein and its native ligand is evaluated using the RMSD (root mean square deviation) value. An RMSD value of less than 2 Å($< 2$ Å) is considered acceptable and indicates good reproducibility of the docking result [21].

### Preparation of 3D structure of serotonin test ligand

The preparation and optimization of the test ligand, serotonin, were carried out using Chem3D and AutoDock tools software [19]. The 3D structure of serotonin was prepared through energy minimization using the force field molecular mechanism (MM2) method [22]. The resulting minimized structure was saved in `.pdb` format. Subsequently, the test ligand structure was optimized by adding hydrogen atoms, followed by a non-polar merge. Gasteiger charges were then applied to the ligand structure. The final step involved adjusting the torsional rotation points using the same procedure applied to the native *l*-norepinephrine ligand.

The fully optimized structure of the serotonin ligand was saved in `.pdbqt` format.

### Molecular docking between D7 protein and serotonin test ligand

The process of molecular docking of D7 protein with the serotonin test ligand is carried out using AutoDock tools software. The first step of this process is to place the structure file of D7 protein in `.pdbqt` format and the structure file of the serotonin ligand into the same folder. This folder also contains the programs AutoGrid4 and AutoDock4 [23].

The D7 protein was first set up as the macromolecule, and the serotonin ligand configuration was used to determine and create the gridbox. The gridbox used is based on the result of coordinate adjustments from the validation or re-docking process between D7 protein and the native ligand *l*-norepinephrine. The coordinate adjustments of the gridbox on protein D7 with the test ligand serotonin are then saved in `.gpf` format in the same folder. The next step is to run the AutoGrid4 program using the Command Prompt (CMD). This program is executed based on the coordinate settings of the gridbox that have been created. The next step is to run the AutoDock4 program, which treats the protein as rigid. The AutoDock4 program is run with the same command based on the previous AutoGrid data [24].

### Analysis and visualization of molecular docking results between D7 protein and serotonin test ligand

The analysis of the molecular docking results between D7 protein and the serotonin test ligand is shown from several parameters such as the Gibbs free energy value ($\Delta G$), types of chemical bonds, and amino acid residues involved in the interactions formed. The results of the Gibbs free energy values were compared with the validation results of the re-docking of D7 protein with the native ligand *l*-norepinephrine. This comparison was made to observe the differences in Gibbs free energy in the formation of interactions between D7 protein and the test ligand.

**Table 1.** Properly formatted D7 protein sequence of *Ae. aegypti*

| |
|---|
| `>sp\|P18153\|ALL2_AEDAE 37 kDa salivary gland allergen` |
| `Aed a 2 OS=Aedes aegypti OX=7159 GN=D7 PE=1 SV=2` |
| `MKEDTLAAVIFSVVASTGPFDPEEMLFTFTRCMEDNLLEDGPNRLPMLAKW` |
| `KEWINEPVDSPATQCGFKCVLVRTGLYDPVAQKFDASVIQEGFKAYPSLG` |
| `EKSKVEAYANAVQQLPSTNNDCAAVFKAYDPVHKAHKDTSKNLFHGNKEL` |
| `TKGLYEKLGKDIROKKQSYFEECENKYYPAGSDKRQQLCKIROYTVLDDA` |
| `LFKEHTDCVMKGIRYITKNNELDAEEVKRDEMQVNKDTKALEKVLNDCKS` |
| `KEPSNAGEKSWHYXKCLVSSVKDDEKEAFDYREVKSQIYAFNLPKKQVYS` |
| `KPAVQSQVMEIDGKQCPQ` |

The results of the molecular docking were then visualized to observe the conformation of the bond between the 3D structure and the two-dimensional structure of the protein and test ligand. The visualization results can include information on amino acid residues and the types of bonds formed. Visualization and analysis of the interaction between D7 protein and the serotonin test ligand were performed using BIOVIA discovery studio software [25].

## Results and Discussions

### 3D structure of D7 protein from *Ae. aegypti* salivary gland

The amino acid sequence data of the D7 protein from the *Ae. aegypti* salivary gland was obtained from the UniProt database with accession number P18153. The protein is identified by the gene name D7 and is known as the salivary gland allergen Aed a 2, with a molecular weight of 37 kDa. The D7 protein with accession number P18153 is a monomeric protein with a long chain domain that has binding affinity for a ligand. The protein is composed of 321 amino acid residues, as shown in **Table 1**. The 3D structure of the D7 protein from *Ae. aegypti* was obtained using homology modeling techniques through the SWISS-MODEL protein database. Construction of the 3D protein structure was carried out using amino acid sequence data of the D7 protein from the salivary glands of *Ae. aegypti*. Based on the homology modeling process, the best model selected used the template with PDB id `3dye.1`. The information on the 3D structure model of the protein is known as the D7 protein crystal structure of the AeD7–norepinephrine complex.

The 3D structure of the D7 protein contains a norepinephrine molecule which will then be used as a native ligand. The 3D structure of the D7 protein shows two domains of the polypeptide chain with different functions. The N-terminal domain can bind cysteinyl leukotriene molecules, while the C-terminal domain can bind biogenic amine molecules [10]. Visualization of the 3D structure model of protein D7 was carried out using the X-ray crystallography method with a resolution of 1.75 Å. The 3D structure model of a protein is said to have good quality if it shows a resolution of < 2.5 Å from the results of X-ray crystallography [20]. The 3D structure model of the D7 *Ae. aegypti* protein can be seen in **Figure 1**.

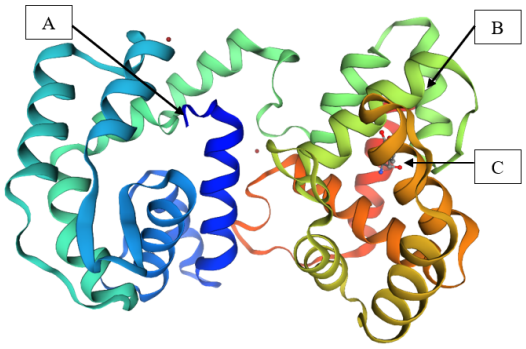



**Figure 1.** 3D structure of D7 protein (STML ID: 3dye.1), **A:** domain terminal-N, **B:** domain terminal-C, **C:** native LNR ligand binding site

### Assessment of the structural quality of the *Ae. aegypti* salivary gland D7 protein model

Data from the results of homology modeling of D7 protein were obtained from the UniProt website with the accession code P18153. The sequence obtained was then copied to the SWISS-MODEL website to build the model. The results of the model build obtained the template PDB id 3dye.1. The results of homology modeling were obtained with crystallography results of 1.75 Å using the X-ray method. For crystallography, a smaller value indicates better resolution [26]. Assessment of homology modeling results is based on several parameters such as GMQE value, QMEAN value, QMEANDisCo value, and sequence identity [27].

The GMQE (global model quality estimate) value parameter is a value that describes the quality of the alignment between the target and template. The range for the GMQE value means that if it approaches one, it indicates a higher level of accuracy of the protein model [28]. The value of GMQE is expressed with a range of values between 0 and 1. The value of the D7 protein model on the GMQE value parameter is 0.91, where the results indicate that the accuracy of the model is good. **Table S1** shows the results of the model quality assessment on the D7 protein.

The QMEAN value is a composite score of a combined assessment that can determine an estimate of the global absolute quality (entire structure) and local (per amino acid residue) based on a single model. The QMEAN score ranges from 0 to 1, where a value of 1 means good [29]. This QMEAN value can be represented by the Z-score value. The Z-score value at the model position (marked with a red asterisk) in the Z-value distribution. This result is marked in **Figure 2**. The red asterisk indicates the model's Z-score is within the typical range for native proteins of similar size, suggesting reasonable overall quality. Estimates of the local quality of the model per amino acid residue can also help in providing an explanation regarding the quality of the D7 protein to be used. This Z-score reflects the overall model quality (QMEAN).

The sequence identity of the D7 protein model results has a value of 95.70%. Sequence identity is a value that can indicate the percentage of residues in the target protein sequence that are identical to those in the template protein sequence [30]. The value of the acceptable sequence identity starts at 30%, where the higher value indicates the level of accuracy between the target protein and the template protein.

The results based on the Ramachandran plot in **Figure 3**, the protein has good structural quality if the amino acid residues are mostly in the favored area rather than the outliers. Lighter shades or white indicate residues with less favorable conformations. The Φ sign is the phi dihedral angle and the Ψ sign is the psi dihedral angle, where both represent the dihedral angles of the amino acid residue backbone. The Ramachandran plot shows residues primarily in the allowed regions. The results of the D7 protein parameters in the Ramachandran plot show residues distributed across the favored and allowed regions, indicating a good and

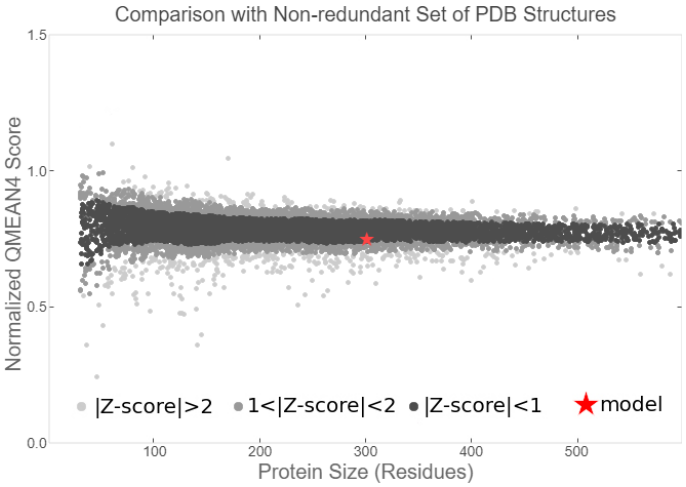



**Figure 2.** Model position (star) on Z Score of protein D7

stable structure. Protein D7 shown in **Table 2** has a favored area of 98.66% and an outlier area of 0.00%, which means the structure of the model is very good. The plot uses contour lines to indicate regions of probability density; 98.66% of residues fall within the favored regions (darkest contours), and 0.00% are in outlier regions **Table 2**, indicating very good stereochemical quality.

**Table 2.** Ramachandran Plot Parameters of D7 protein

| Parameter | Model-1 |
|---|---|
| MolProbity Score | 0.65 |
| Clash Score | 0.41 |
| Ramachandran Favoured | 98.66% |
| Ramachandran Outliers | 0.00% |
| Rotamer Outliers | 0.00% |
| C-beta Deviations | 0 |
| Bad Bonds | 0/2504 |
| Bad Angles | A276 ASP, (A296 LEU-A297 PRO), A163 ASP, A140 ASP, (A132 ASP-A133 PRO), (A304 LYS-A205 PRO), A264 HIS, A135 HIS, A207 HIS, A138 HIS, A263 TRP |

The MolProbity score of Model-1 is 0.65. The MolProbity score is a combination of the log-weighted clash score, the percentage of unfavorable Ramachandran outliers, and the percentage of bad side chain rotamers. This score indicates a value that is expected to describe the equivalent resolution of a comparable experimental structure. A lower MolProbity score generally indicates better quality. If the score is lower than typical for structures at the template's resolution (1.75 Å), then the model

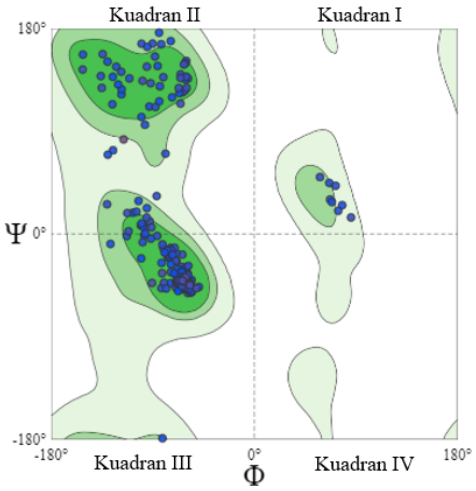



**Figure 3.** Ramachandran Plot of *Aedes aegypti* salivary gland D7 protein

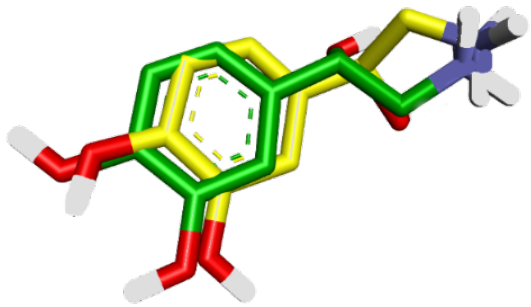


**Figure 4.** Visualization of the overlapping conformation between the native ligand *l*-norepinephrine from crystallography (green) and the conformation of the native ligand *l*-norepinephrine from re-docking (yellow).

quality is considered very good. The resolution of the template crystallography is 1.75 Å.

### 3D structure of native ligand *l*-norepinephrine and test ligand serotonin

The 3D structure model of D7 protein with template PDB id `3dye.1` has a native ligand in the form of *l*-norepinephrine. The *l*-norepinephrine molecule is a type of biogenic amine which is a component of monoamine neurotransmitters [31]. The *l*-norepinephrine molecule is a non-polymer molecule with a molecular weight of 169.178 g/mol. *L*-norepinephrine has a structure containing an aromatic ring and a carbon chain with the chemical formula C8H11NO3. The structure of *l*-norepinephrine consists of 23 atoms, each of which is connected by chemical bonds and has one aromatic ring [32]. The use of native ligands is important for the validation stage of the molecular docking method as well as for determining the orientation of the binding site for test ligands in the molecular docking process [33].

The test ligand used in this study was the serotonin molecule, which is one of the biogenic amines in the human bloodstream. Serotonin in the human body plays a role in the platelet aggregation process through the activation mechanism between platelet cells [34]. The structure of serotonin can interact with functional groups to form hydrogen bonds and can also participate in aromatic interactions [35]. Serotonin (also known as enteramine) has the chemical formula C10H12N2O with a molecular weight of 176.21 g/mol. Serotonin is composed of 25 atoms connected by chemical bonds and has an indole ring system [36]. The differences in the 2D and 3D structures of the *l*-norepinephrine ligand and the serotonin test ligand can be seen in **Table 3**.

### Molecular docking validation method

Validation of the molecular docking method was carried out through the re-docking technique using the native ligand and D7 protein as the target protein in this study. Before validating the molecular docking method, it is necessary to prepare and optimize the structure of the D7 protein by separating it from the native ligand *l*-norepinephrine and removing water molecules, which are non-standard residues. The separation of the native ligand from the target protein structure aims to provide a binding pocket for the test ligand and the target protein. The removal of water molecules from the target protein structure is carried out so that they do not become an obstacle during the molecular docking process. This step is important so that in the molecular docking process, only the ligand structure interacts with the target protein [37].

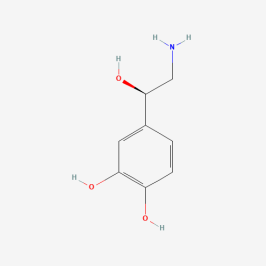
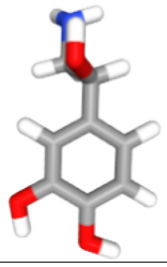
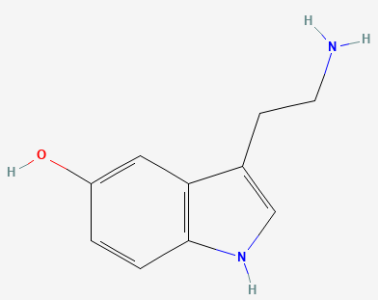
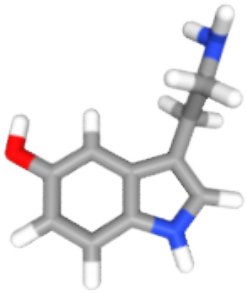
The results of the molecular docking method validation process can be seen from the RMSD value. The RMSD parameter can show the results of the conformational alignment between the native ligand pose resulting from the re-docking process and the native ligand conformation from X-ray crystallography [21]. The RMSD value is obtained by examining the overlap between the conformation of the native ligand resulting from re-docking and the native ligand in its original crystallographic conformation. A smaller RMSD value indicates that the predicted binding pose from re-docking closely matches the experimentally observed binding pose. The validity of the RMSD value is indicated by a value < 2 Å [38].

The RMSD value of the re-docking process between the *l*-norepinephrine ligand and the D7 protein shows a value of 1.088 Å. These results indicate that the validation of the molecular docking method is accepted and the method can be used for the molecular docking process between the D7 protein and the serotonin ligand. The results of the alignment or overlapping conformation between the crystallographic pose (green) and the re-docked pose (yellow) of the native *l*-norepinephrine ligand can be seen in **Figure 4**.

### Molecular docking of protein D7 from *Ae. aegypti* and serotonin test ligand

The molecular docking process is carried out using serotonin ligands that have been prepared and optimized against the D7

**Table 3.** Differences in 2D and 3D structure of native ligands and test ligands

| Ligand | 2D | 3D |
|---|---|---|
| [l]Native ligand (*l*-norepinephrine) |  |  |
| [l]Test ligand (serotonin) |  |  |

protein from *Ae. aegypti*. The grid box coordinates obtained from the re-docking stage with the native ligand are then used as the basis for the grid box for the molecular docking process between the D7 protein and the serotonin ligand. This is done so that the test ligand can bind to the active site of the target protein [39]. In the molecular docking process, the structure of the D7 protein is kept rigid during the docking process. The structure of the serotonin ligand is treated as flexible. This is done so that the test ligand can interact and bind in the most stable conformation within the active site of the amino acid residues of the target protein [40].

### Analysis and visualization of molecular docking results of *Ae. aegypti* D7 protein with serotonin ligand

A $\Delta G$ value > 0 indicates that the binding reaction between the target protein and the test ligand cannot occur spontaneously. Conversely, if the $\Delta G$ value < 0, it indicates that the binding reaction between the target protein and the test ligand occurs spontaneously (a reaction that favors product formation). A $\Delta G$ value = 0 indicates that the reaction is at equilibrium. The more negative the $\Delta G$ value, the stronger the binding affinity between the target protein and the ligand [41]. In addition, the negative value of $\Delta G$ indicates that the interaction between the target protein and the ligand binding process is thermodynamically favorable. This means that the binding between the ligand and the active site of a target protein occurs in a stable condition and spontaneously [42]. The results of molecular docking between protein D7 and serotonin ligand show a $\Delta G$ value of -9.25 kcal/mol. These results indicate that protein D7 can bind to serotonin ligands in a stable and spontaneous manner. This can be correlated with the mechanism during the blood feeding process, where protein D7 from the salivary glands of *Ae. aegypti* can inhibit platelet aggregation by binding biogenic amines, including serotonin, thereby inhibiting serotonin's role in platelet aggregation and disrupting the host's homeostasis reaction [5].

The D7 protein from *Ae. aegypti* has been studied for its potential role in modulating host hemostasis, primarily through its ability to bind biogenic amines and eicosanoids, thereby facilitating blood feeding. However, direct experimental evidence demonstrating its specific function as an inhibitor of platelet aggregation is limited. In contrast, studies on the D7 protein from *Aedes albopictus*, a related mosquito species, have provided functional evidence of its role in inhibiting platelet aggregation. For instance, AlboD7L1, a long-form D7 protein from *Aedes albopictus*, has been shown to bind various ligands and inhibit platelet aggregation in *ex vivo* experiments [10].

Visualization of molecular docking results was carried out to determine the binding interaction mode between protein D7 and serotonin ligand. The target protein can bind to the test ligand through several amino acid residues [20]. The interaction between the target protein and the test ligand is indicated by the formation of several types of chemical bonds, such as hydrogen bonds, hydrophobic interactions, electrostatic interactions, and

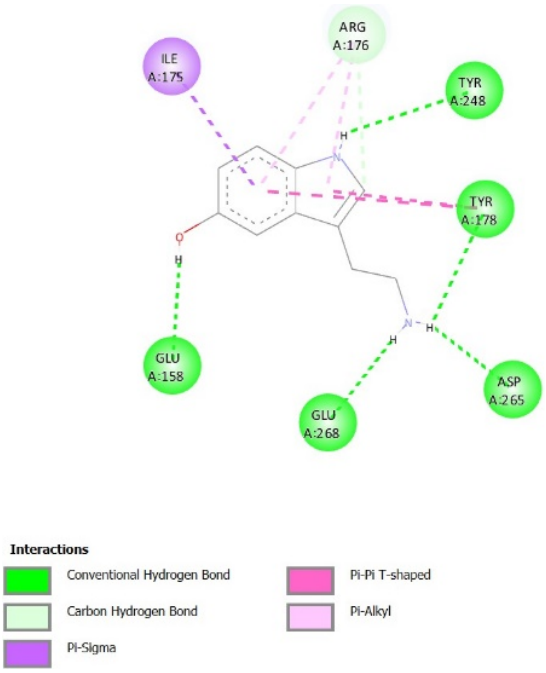



**Figure 5.** Visualization of the interaction of amino acid residues of D7 protein with serotonin ligand.

Van der Waals bonds [43]. The results of molecular docking visualization between protein D7 and serotonin ligand can be seen in **Figure 5**.

**Table 4.** Amino acid residue D7 protein that binds to the serotonin ligand

| Amino acid residue D7 protein | Serotonin ligand atom | Chemical bond |
|---|---|---|
| Glutamic Acid 158 (GLU 158)<br>Tyrosine 178 (TYR 178)<br>Tyrosine 248 (TYR 248)<br>Aspartic Acid 265 (ASP 265)<br>Glutamic Acid 268 (GLU 268) | H (Hydrogen) | Conventional hydrogen bond |
| Arginine 176 (ARG 176) | C (Carbon) | Carbon hydrogen bond |
| Isoleucine 175 (ILE 175) | C-H (Carbon Hydrogen) | $\pi$-sigma bond |
| Tyrosine 178 (TYR 178) | Pi-Orbitals | $\pi$-$\pi$ T-shaped bond |
| Arginine 176 (ARG 176) | Pi-Orbitals | $\pi$-Alkyl bond |

**Table 4** shows the various types of chemical bonds formed between amino acid residues of the D7 protein that interact with atoms on the serotonin ligand. The chemical bonds formed are interactions that occur at the active site of the D7 protein binding to the serotonin test ligand. Amino acid residues on the active site of the D7 protein that bind and interact with the serotonin ligand include GLU 158, ILE 175, ARG 176, TYR 178, TYR 248, ASP 265, and GLU 268. The chemical bonds formed in the interaction between the D7 protein and the serotonin ligand include conventional hydrogen bonds, carbon-hydrogen bonds, $\pi$-sigma bonds, $\pi$-$\pi$ T-shaped bonds, and $\pi$-alkyl bonds. Conventional hydrogen bonds are formed involving several amino acid residues: GLU 158, TYR 178, TYR 248, ASP 265, and GLU 268. The carbon-hydrogen bond formed involves one amino acid residue, namely ARG 176. The $\pi$-sigma bond formed only involves one amino acid residue, namely ILE 175. The $\pi$-$\pi$ T-shaped bond formed also involves one amino acid residue, namely TYR 178. The $\pi$-alkyl bond also involves one amino acid residue, namely ARG 176.

Hydrogen bonds are formed between hydrogen atoms and electronegative atoms. This hydrogen bond formation is related to binding energy ($\Delta G$). The formation of hydrogen bonds can release energy due to covalent interactions, resulting in a negative change in enthalpy ($\Delta H$). A negative change in enthalpy can occur when a protein and ligand bind. This result can lead to a negative $\Delta G$ value, meaning the binding occurs spontaneously or is stable [44]. The interaction between the serotonin ligand and protein D7 involved in hydrogen bonding consists of conventional hydrogen bonds. In conventional hydrogen bonds, hydrogen is shared between electronegative atoms acting as donors (e.g., O-H, N-H on the ligand or protein) and acceptors (e.g., O, N on the protein or ligand) [45].

Hydrogen bonds significantly influence the stability of the D7 protein-ligand complex because both the protein and ligand contain potential hydrogen bond donors (like N-H and O-H) and acceptors. These structures act as donors or acceptors in hydrogen bonds. The next type is the $\pi$-sigma bond, which is an interaction involving the $\pi$-system of an aromatic ring and a sigma bond (like C-H) [46]. The $\pi$-sigma bond occurs between the amino acid ILE 175 and the ligand. The next bond is a $\pi$-$\pi$ T-shaped bond, which is an electron interaction between two aromatic groups but in a T shape [47]. In this geometry, the edge of one aromatic ring points towards the face of the other aromatic ring. The last interaction is a type of $\pi$-alkyl bond, which is an interaction between electrons from the aromatic group and the electron group from the alkyl group [47].

## Conclusions

The molecular docking results show that there is an interaction between the D7 protein from the salivary gland of *Ae. aegypti* (accession number P18153) and the serotonin test ligand (accession number 5202). The interaction between the D7 protein and the serotonin test ligand shows stability and spontaneity based on the $\Delta G$ value of -9.25 kcal/mol. Based on the $\Delta G$ parameter, this indicates that the D7 protein and the serotonin ligand can bind spontaneously and stably. The amino acid residues of protein D7 that interact with the serotonin ligand atoms include GLU 158, ILE 175, ARG 176, TYR 178, TYR 248, ASP 265, and GLU 268. Thus, the D7 protein from the salivary gland of *Ae. aegypti* has potential as a new agent for platelet aggregation inhibition for drug discovery and development in the fields of health and pharmacy.

## Supplementary

Table S1: D7 protein model quality assessment parameters.

## Reference


1. Wathon S, Mutiah F, Oktarianti R, Senjarini K. Purifikasi protein imunogenik 31 dan 56 kDa dari kelenjar saliva *Aedes aegypti*. Jurnal Bioteknologi dan Biosains Indonesia. 2020;7(1):59-71.
2. Wathon S, Oktarianti R, Senjarini K. Kelenjar saliva *Aedes aegypti* harapan baru pengembangan vaksin demam berdarah dengue. 2016.
3. Guerrero D, Cantaert T, Missé D. *Aedes* mosquito salivary components and their effect on the immune response to arboviruses. Frontiers in Cellular and Infection Microbiology. 2020;10:407.
4. Barros MS, Lara PG, Fonseca MT, Moretti EH, Filgueiras LR, Martins JO, et al. *Aedes aegypti* saliva impairs M1-associated proinflammatory phenotype without promoting or affecting M2 polarization of murine macrophages. Parasites & Vectors. 2019;12:1-15.
5. Gavor E, Choong YK, Liu Y, Pompon J, Ooi EE, Mok YK, et al. Identification of *Aedes aegypti* salivary gland proteins interacting with human immune receptor proteins. PLoS Neglected Tropical Diseases. 2022;16(9):e0010743.
6. Chowdhury A, Modahl CM, Missé D, Kini RM, Pompon J. High resolution proteomics of *Aedes aegypti* salivary glands infected with either dengue, Zika or chikungunya viruses identify new virus specific and broad antiviral factors. Scientific Reports. 2021;11(1):23696.
7. McCracken M, Christofferson R, Grasperge B, Calvo E, Chisenhall D, Mores C. *Aedes aegypti* salivary protein aegyptin co-inoculation modulates dengue virus infection in the vertebrate host. Virology. 2014;468:133-9.
8. Gulley MM, Zhang X, Michel K. The roles of serpins in mosquito immunology and physiology. Journal of insect physiology. 2013;59(2):138-47.
9. Alvarenga PH, Andersen JF. An overview of D7 protein structure and physiological roles in blood-feeding nematocera. Biology. 2022;12(1):39.
10. Martin-Martin I, Smith LB, Chagas AC, Sá-Nunes A, Shrivastava G, Valenzuela-Leon PC, et al. *Aedes* albopictus D7 salivary protein prevents host hemostasis and inflammation. Biomolecules. 2020;10(10):1372.
11. Michael ZM. Obat Penginduksi Pendarahan. Program Studi Apoteker, Fakultas Farmasi Universitas Padjadjaran. 2017.
12. Prasetiawati R, Suherman M, Permana B, Rahmawati R. Molecular docking study of anthocyanidin compounds against Epidermal Growth Factor Receptor (EGFR) as antilung cancer. Indonesian Journal of Pharmaceutical Science and Technology. 2021;8(1):8-20.
13. Wathon S, Oktarianti R, Senjarini K. Molecular Docking of Interaction between D7 Protein from the Salivary Gland of *Aedes aegypti* and Leukotriene A4 for Developing Thrombolytic Agent. In: BIO Web of Conferences. vol. 101. EDP Sciences; 2024. p. 04002.
14. Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic acids research. 2019;47(D1):D506-15.
15. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic acids research. 2018;46(W1):W296-303.
16. Bienert S, Waterhouse A, De Beer TA, Tauriello G, Studer G, Bordoli L, et al. The SWISS-MODEL Repositorynew features and functionality. Nucleic acids research. 2017;45(D1):D313-9.
17. Wang Y, Bryant SH, Cheng T, Wang J, Gindulyte A, Shoemaker BA, et al. Pubchem bioassay: 2017 update. Nucleic acids research. 2017;45(D1):D955-63.
18. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry. 2009;30(16):2785-91.
19. Huey R, Morris GM, Forli S, et al. Using AutoDock 4 and AutoDock vina with AutoDockTools: a tutorial. The Scripps Research Institute Molecular Graphics Laboratory. 2012;10550(92037):1000.
20. Sari IW, Junaidin J, Pratiwi D. Studi Molecular Docking Senyawa Flavonoid Herba Kumis Kucing (Orthosiphon Stamineus B.) Pada Reseptor A-Glukosidase Sebagai Antidiabetes Tipe 2. Jurnal Farmagazine. 2020;7(2):54-60.
21. Sari IW, Junaidin J, Pratiwi D. Studi Molecular Docking Senyawa Flavonoid Herba Kumis Kucing (Orthosiphon Stamineus B.) Pada Reseptor A-Glukosidase Sebagai Antidiabetes Tipe 2. Jurnal Farmagazine. 2020;7(2):54-60.
22. Allinger NL. Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms. Journal of the American Chemical Society. 1977;99(25):8127-34.
23. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 2010;31(2):455-61.
24. Endriyatno NC, Walid M. Studi In Silico Kandungan Senyawa Daun Srikaya *(Annona squamosa* L.) Terhadap Protein Dihydrofolate Reductase Pada Mycobacterium tuberculosis. Pharmacon: Jurnal Farmasi Indonesia. 2022;19(1):87-98.

25. Baroroh U, Biotek M, Muscifa ZS, Destiarani W, Rohmatullah FG, Yusuf M. Molecular interaction analysis and visualization of protein-ligand docking using Biovia Discovery Studio Visualizer. Indonesian Journal of Computational Biology (IJCB). 2023;2(1):22-30.

26. Wijaya H, Hasanah F. Prediction of Three-dimensional Structure From Food Allergen Protein Through Homology Method Using Swiss-model Program. Biopropal Industry. 2016;7(2):83-94.

27. Ahmed MZS. Homology Modeling and Structural Analysis of of the Flavanone 3-Hydroxylase (F3H) and Flavonoid 3-hydroxylase (F3H) Genes from Ginkgo Biloba (L.).

28. Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, Schmidt T, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic acids research. 2014;42(W1):W252-8.

29. Komari N, Hadi S, Suhartono E. Protein Modeling with Homology Modeling using SWISS-MODEL: Protein Modeling with Homology Modeling using SWISS-MODEL. Journal of Mathematics and Science Network. 2020;2(2):65-70.

30. Kanduc D. Homology, similarity, and identity in peptide epitope immunodefinition. Journal of Peptide Science. 2012;18(8):487-94.

31. Wu F, Liang T, Xiao W, Wang T. Norepinephrine in goal-directed fluid therapy during general anesthesia in elderly patients undergoing spinal operation: determining effective infusion rate to enhance postoperative functions. Current Genomics. 2021;22(8):620-9.

32. Angles R, Arenas-Salinas M, García R, Ingram B. An optimized relational database for querying structural patterns in proteins. Database. 2024;2024:baad093.

33. Aziz A, Andrianto D, Safithri M. Penambatan Molekuler Senyawa Bioaktif Daun Wungu (*Graptophyllum Pictum* (L) Griff) sebagai Inhibitor Tirosinase. Indonesian Journal of Pharmaceutical Science and Technology. 2022;9(2):96-107.

34. Marcinkowska M, Kubacka M, Zagorska A, Jaromin A, Fajkis-Zajaczkowska N, Kolaczkowski M. Exploring the antiplatelet activity of serotonin 5-HT2A receptor antagonists bearing 6-fluorobenzo [d] isoxazol-3-yl) propyl) motif–as potential therapeutic agents in the prevention of cardiovascular diseases. Biomedicine & Pharmacotherapy. 2022;145:112424.

35. Yang D, Gouaux E. Illumination of serotonin transporter mechanism and role of the allosteric site. Science Advances. 2021;7(49):eabl3857.

36. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic acids research. 2023;51(D1):D1373-80.

37. Attique SA, Hassan M, Usman M, Atif RM, Mahboob S, Al-Ghanim KA, et al. A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension. International journal of environmental research and public health. 2019;16(6):923.

38. Ferencz L, Muntean DL. Identification of new superwarfarin-type rodenticides by structural similarity. The docking of ligands on the vitamin K epoxide reductase enzymes active site. Acta Universitatis Sapientiae, Agriculture and Environment. 2015;7(1):108-22.

39. Morris GM, Goodsell DS, Pique ME, Huey R, Hart WE, Halliday S, et al.. AutoDock Version 4.2: Updated for version 4.2.6. Automated Docking of Flexible Ligands to Flexible Receptors; 2014. The Scripps Research Institute, La Jolla, CA, USA.

40. Khan T, Lawrence AJ, Azad I, Raza S, Khan AR. Molecular Docking Simulation with Special Reference to Flexible Docking Approach. JSM Chemistry. 2018;6(1):1-5.

41. Jiménez JS, Benítez MJ. Gibbs Free Energy and Enthalpy–Entropy Compensation in Protein–Ligand Interactions. Biophysica. 2024;4(2):298-309.

42. Choma CT. Characterizing Binding Interactions by ITC; 2006. Document No. 10111020706.

43. Forouzesh N, Mishra N. An Effective MM/GBSA Protocol for Absolute Binding Free Energy Calculations: A Case Study on SARS-CoV-2 Spike Protein and the Human ACE2 Receptor. Molecules. 2021;26(8):2383.

44. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, et al. Insights into Protein–Ligand Interactions: Mechanisms, Models, and Methods. International Journal of Molecular Sciences. 2016;17(2):144.

45. Mishra KK, Borish K, Singh G, Panwaria P, Metya S, Madhusudhan MS, et al. Observation of an Unusually Large IR Red-Shift in an Unconventional S–H$\cdots$S Hydrogen-Bond. Journal of Physical Chemistry Letters. 2021;12(4):1228-35.

46. Zierkiewicz W, Michalczyk M, Scheiner S. Noncovalent Bonds through Sigma and Pi-Hole Located on the Same Molecule. Guiding Principles and Comparisons. Molecules. 2021;26(6):1740.

47. Alencar WLM, da Silva Arouche T, Neto AFG, de Castro Ramalho T, de Carvalho Júnior RN, de Jesus Chaves Neto AM. Interactions of Co, Cu, and Non-metal Phthalocyanines with External Structures of SARS-CoV-2 Using Docking and Molecular Dynamics. Scientific Reports. 2022;12(1):3316.