Homology Modeling, Molecular Dynamics Simulation and Essential Dynamics on *Anopheles gambiae* D7r1

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Abstract The proteins produced by the saliva and salivary glands of blood sucking arthropods play a vital role in transmission of the infected parasite to host and interfere the parasitic life cycle. The structure prediction of D7r1 and its active site leads to inhibition of hemostasis and inflammation in the host. D7r1 is a member of D7-related (D7r) salivary gland proteins that interferes various aspects of host physiology. Considering the significance of a protein, three dimensional structure of D7r1 model was generated by homology modeling and validated by PROCHECK, ERRAT, Verify-3D, RAMPAGE and Q mean server. The predicted structure has 96.9% of residues in the most favored region of the Ramachandran plot. The sequence and structural alignment between D7r1 and template 2PQL reveals that similar active site residues such as Ile42, Arg43, Tyr45, His56, Met57, Val60, Phe131, and Met156 involved in binding pocket formation. Further, a molecular dynamics simulation study was performed to reveal the prolonged stability of D7r1 protein. The essential dynamics which include PCA and FEL analysis were used to evaluate the conformational stability of D7r1. This combined molecular dynamics simulation and essential dynamics were used to provide comprehensive information of D7r1 and its active site prediction gain insights into the development of novel lead molecules for disrupting host-seeking behavior of mosquitoes.

Keywords *Anopheles gambiae*, D7-related (D7r) Proteins, Homology Modeling, Molecular Dynamics Simulation, PCA and FEL Analysis

burden in developing countries [1]. In 2016, World Health Organization (WHO) reported the death of 438,000 people out of 214 million people with malarial infection. The deceased people were mainly children under the age of 5 [2]. The African mosquito *Anopheles gambiae*, carries the pathogen *Plasmodium falciparum* in their saliva and responsible for transmitting malaria to humans. The multi-drug resistance capability of *Plasmodium* parasites as well as resistance against chemotherapeutic agents makes it imperative to develop new anti-malarial drug agents [3]. Researchers are currently focused on developing new drugs and vaccines against the parasite. Till date, the most successful tactic to control malarial transmission to humans is found to be a reduction of the frequency of contact between the mosquito vectors and their human targets [4].

The African mosquito *Anopheles gambiae* is considered the best studied malarial parasite of *Plasmodium falciparum* and also plays a crucial role in the parasite life cycle. Approximately, 450 species of *Anopheles* mosquitoes are available worldwide, among them 60 species are reported to be a vector of human malaria [5]. Parasites are transmitted to humans once *Anopheles* mosquito bites and subsequently destroy red blood cells by multiplying in the host. Predominantly, female *Anopheles gambiae* mosquitos have an extremely high preference for feeding on humans. The selectivity of the female mosquito for human host is guided by olfactory responses to odor molecules that emanate from human skin and sweat [6]. The salivary gland of blood-feeding arthropods contains various potent bioactive substances essential for blood feeding. Several proteins produced by the salivary glands are significant for overcoming the problems caused by the host such as itch responses, immune defenses and homeostasis [7]. Further, it has been revealed that the pharmacological activity of arthropod salivary interrupts malarial pathogen transmission. Proteomic studies were performed on mosquito salivary gland proteins to define their imperative role on *Plasmodium falciparum* [8].

The identification of 67 proteins from *Anopheles gambiae*

1. Introduction

The blood feeding insects are responsible for transmitting a number of potentially lethal diseases like encephalitis, yellow fever and malaria (a most deadly tropical disease) are considered to be an important reason for socio-economic

salivary glands by Kalume et al. [9] has been an initial step towards the grouping of numerous proteins and peptides in the salivary proteome. Earlier studies reports that the saliva from female *Anopheles gambiae* has several antihemostatic proteins that include the platelet aggregation inhibitor apyrase, anophelin, and catechol oxidase-peroxidase. Of these, the D7-related (D7r) proteins are known to interfere with various aspects of host physiology and are divided into a group of five polypeptides (D7r1-D7r5) [10]. Further, recent study confirms that five major salivary gland proteins such as a putative 5'-nucleotidase, anti-platelet protein, long form D7 salivary protein, D7r1 and gSG6 from *An. campestris* mosquitoes involved in blood feeding [11]. Since, saliva proteins are antigenic and immunogenic which enhances the infectivity of the parasite and its immunomodulatory, anti-inflammatory and anti-coagulant properties helps in successful blood feeding [12].

D7r1 protein also known as hamadarin, exhibits anticoagulant or antikinin activity by forming strong interactions with high molecular weight kininogen and factor XIIa, both are considered to be important components of the contact activation system of coagulation [3, 13]. Moreover, It has been proven that D7r (D7r1-D7r4) proteins bind to biogenic amines such as serotonin, norepinephrine and histamine, accordingly reduces the concentrations of these effectors at blood feeding site [3]. Surprisingly, D7r5 protein is not involved in any strong binding interaction with biogenic amines. The sequence comparison among D7r protein families (D7r1-D7r5) illustrates a possible relationship among the D7r proteins and may function as scavengers of biogenic amines [14].

Owing to the importance of D7r1 as a potent antimalarial drug target, the comparative based homology modeling was performed to generate 3D models of D7r1 by using MODELLER 9v7 suite. Several standalone tools and servers were used to evaluate the reliability of predicted model and indicating the high quality of the model. Further, the modeled structure was minimized and equilibrated by Molecular Dynamics simulation (MDS) for period of 50 ns to ensure the structure stability. Additionally, the Principal Component Analysis (PCA) and Free Energy Landscape (FEL) were used to investigate the functional dynamics and conformational changes in D7r1 protein.

2. Materials and Methods

2.1. Protein Sequence Information

The primary protein sequence of D7r1 protein is retrieved from the Uniprot database (UniProtKB accession number: Q9UB30) in FASTA format. The ProtParam tool in the ExPASy server [15] was used to calculate the physio-chemical properties of D7r1. The secondary structure of D7r1 is predicted by PSIPRED [16] and analyzed by SOPMA server [17].

2.2. Disordered Region Prediction

The high flexibility and disordered regions of D7r1 protein were determined by GlobPlot [18].

2.3. Homology Modeling of D7r1

Comparative modeling or Homology modeling has become an efficient approach for predicting the three dimensional structure of an unknown protein based on template sequence alignment. The homology modeling of D7r1 was carried out by MODELLER (9v11) suite [19] and the alignment of target and template proteins were performed by Clustal Omega [20]. PyMOL [21] and Chimera [22] were used for visualization.

2.4. Structure Validation of D7r1

The stereo chemical validation and the reliability of predicted model were carried out using various online tools. RAMPAGE [23] server, assessed the stereo chemical quality of protein by building Ramachandran Plot of the protein backbone structure. The structure was further subjected to Structural Analysis and Verification Server (SAVES) [24] which includes PROCHECK [25] and ERRAT [26] to evaluate the reliability of protein. In addition, the structure was given to Q MEAN (Qualitative Model Energy ANALysis) [27] server to calculate the overall quality of the protein by comparing experimentally solved reference structures. The Z-score calculated by ProSA [28] web server is used to inspect the overall quality of the protein. Further, the quantitative evaluation of computationally modeled D7r1 structure was carried out in PROSESS (PROtein Structure Evaluation Suite and Server) [29] which uses VADAR (Volume Area Dihedral Angle Reporter) [30] web tool to identify global and residue specific problem.

2.5. Molecular Dynamics Simulation

The structural behavior and conformational motion of modeled D7r1 was studied by molecular dynamics simulation using GROMACS package version 4.5.3 [31, 32]. The protein topology was built by GROMOS96 43a1 force field [33] and the protein model was solvated with SPC216 water model in a cubic box size of 1.50 nm. The system was surrounded with simple point charge (SPC) and neutralized by adding 10 chlorine(Cl⁻) ions. The periodic boundary conditions were applied in all directions to avoid edge effects. Prior to MD simulation, the system was subjected to two rounds of energy minimization with maximum steps of 50,000 for the modeled **protein** using steepest descent algorithm, followed by conjugate gradient algorithm with a tolerance of 1000 kJmol⁻¹nm⁻¹. The energy minimized systems was equilibrated at both NVT (constant volume and normal temperature) and NPT (constant pressure and normal temperature) ensembles for 150 ps. The Particle Mesh Ewald

(PME) [34] summation was used for long range electrostatic interactions with a PME order of 4 and a Fourier spacing of 0.16 nm. The LINCS algorithm [32] was used for constraining the bonds containing hydrogen atoms and SETTLE algorithm [35] was used to constrain the geometry of water molecules. V-rescale weak coupling method was used to regulate the temperature (300K) and Parrinello–Rahman method [36] was used to set the pressure (at 1atm). Finally, the MD run of 50 ns time scale was carried out with a time step of 2 femtosecond (fs) and the simulated model was also validated for their structure quality using above mentioned servers.

2.6. Principal Components and Free Energy Landscape Analysis

PCA is an effective method for finding global and correlated motions by obtaining standard information from the MD trajectories through dimensionality reduction method [37, 38]. PCA was used to distinguish the large scale collective motions from the random thermal fluctuations. These large scale collective motions are responsible for determining the functional state of a protein [39].

PCA method is based on the calculation and diagonalization of the covariance matrix. The elements C_{ij} in this matrix is defined by [40].

$$C_{ij} = \langle (x_i - \bar{x}_i) (x_j - \bar{x}_j) \rangle \quad (1)$$

Where, x_i (x_j) is the coordinate of the i th (j th) atom and represents an ensemble average. In general, the few initial principal components (PCs) are responsible for the most important conformational changes of bimolecular system by storing eigenvectors with the highest eigenvalues. The g_covar module of GROMACS package [31, 32] was used to generate and diagonalize the covariance matrix. Meanwhile, g_anaeig module was used to calculate the eigenvalues and eigenvectors. The eigenvectors of the matrix represent the directions of the motions and the eigenvalues describes the magnitude of the motion for each direction. The cosine content (C_i) value of PCs is considered to be an important measure to differentiate the PCs that captured the non-random fluctuations from the collective motions. Normally, the cosine content value varies between 0 (no cosine) and 1 (perfect cosine) in the total simulation time period (T):

$$C_i = \frac{2}{T} \left(\int \cos(i\pi t) p_i(t) dt \right)^2 \left(\int p_i^2(t) dt \right)^{-1}$$

In general, the protein dynamics can be defined based on their initial eigenvector cosine contribution. However, in most of the cases the cosine content value of first eigenvector is near to 1, which normally represents large scale motions in the protein simulation system. Therefore, these large scale motions cannot be used as a measure for describing the protein structural behaviour in terms of the free energy landscape (FEL). The FEL can be constructed on the basis of PCA [40] and provide a quantitative description of protein

dynamics. In this study, PCA was performed in order to investigate the functional motions of D7r1 by extracting the top 20 PCs (Projection eigenvectors). In which, the cosine content less than 0.2 were used as suitable reaction coordinates (PC1 and PC2) to generate the FEL energy graph by the g_sham module of GROMACS. The FEL analysis gives descriptive information on dominant conformations with lowest energy and provides their structural transition during the simulation period.

2.7. Active Site Prediction

Active site, also called catalytic site of protein consisting of amino acid residues act as a donor or acceptor for ligand or lead molecules. Identification and characterization of the protein active site is a crucial step in molecular docking studies. The active site of D7r1 protein was predicted by both computational approach and literature studies. The COACH server which contains COFACTOR [41], ConCavity [42] and S-SITE [43] are used to predict the possible ligand binding site or binding pocket of D7r1 protein.

3. Results and Discussion

3.1. Primary and Secondary Structure Analysis

Table 1. Amino acid composition of D7r1

Amino acid name	Amino acid No	Amino acid percentage (%)
Ala (A)	11	6.7%
Arg (R)	4	2.4%
Asn(N)	6	3.6%
Asp (D)	14	8.5%
C0ys (C)	7	4.2%
Gln (Q)	5	3.0%
Glu (E)	6	3.6%
His (H)	5	3.0%
Ile (I)	6	3.6%
Leu (L)	19	11.5%
Lys (K)	26	15.8%
Met (M)	6	3.6%
Phe (F)	6	3.6%
Pro (P)	6	3.6%
Ser (S)	8	4.8%
Thr (T)	6	3.6%
Trp (W)	0	0.0%
Tyr (Y)	5	3.0%
Val (V)	12	7.3%

The protein sequence of D7r1 (165aa, UniProtKB accession number: Q9UB30) was fetched from Uniprot database [48] in FASTA format. The template based modeling also called homology modeling requires experimentally determined protein structure as a template. The most appropriate template for homology modeling was determined by BLASTp search against the PDB database [49]. The crystal structure of D7r4 (PDB ID: 2PQL) from *Anopheles gambiae* at 2.2 Å resolution [10] was selected as a template based on the maximum identity score and lower *e* value. The D7r1 protein and template 2PQL showed 55% sequence identity and 84% query coverage with an *e* value of 3e-54, which meets the criteria for choosing a suitable template. Clustal Omega was used to perform an alignment between the target and template protein sequences. Structural modeling was performed by MODELLER (9v11) which generates ten models out of which the best structure is chosen based on the lowest DOPE score (-16487.28906).

The structural features of D7r1 model were similar to other D7r protein family members and the predicted model adopted all structural features from the D7r4 template. The superimposition of model D7r1 and template 2PQL shows that the generated model quality was good with a least RMSD value of 0.117 Å. The sequence and structural alignment between the target and template protein reveals that similar active site residues involved in the binding pocket formation (Figure 2). The predicted tertiary structure of D7r1 contains eight helices $\alpha 1$ to $\alpha 8$ which are connected by several loops and the modeled structure given in Figure 3. Among eight helices, $\alpha 5$ and $\alpha 7$ helices found to be larger helices with the helical lengths of 23.97 Å and 26.99 Å respectively; whereas, $\alpha 1$ and $\alpha 4$ helices predicted as small helices with helical lengths of 9.69 Å and 15.92 Å respectively. The N-terminal region of D7r1 has adopted long loop region (M1-T23) with a length of 18.29 Å. The number of α helices and their corresponding amino acid residues with their helical length is given in Table 2.

The protein sequence or primary structure decides the function and stability of the protein. Therefore, it was important to analyze the amino acid sequence which provides basic information about D7r1 protein. The physio-chemical parameters of D7r1 protein were determined by ProtParam tool of ExPASy server. The amino acid composition of D7r1 is given in Table 1. The calculated isoelectric point for D7r1 was 9.24 which suggest the presence of a high number of charged residues. The estimated molecular weight of D7r1 is 18,666 Da and an aliphatic index [44] of 86.85. The extinction coefficient [45] of protein (at 280 nm in measured in water) assuming all pairs of Cys residues from cysteines was $7825 \text{ M}^{-1} \text{ cm}^{-1}$ and reduced form was $7450 \text{ M}^{-1} \text{ cm}^{-1}$. The instability index [46] of D7r1 was computed to be 24.38, which clearly implies that the generated 3D model was more stable because an instability index more than 40 is considered unstable [47]. The stability of protein was further confirmed by a Grand average of hydropathicity (GRAVY) value of -0.353. The

Helix	Start a.a	End a.a	No.of residues	Helical length (Å)	Sequence
1	Val24	Lys30	6	9.69	VKKCEK
2	Ala33	Lys44	12	18.14	ASLKSQ LCEIRK
3	Pro51	Leu64	14	19.69	PDMDKHMDCVMKAL
4	Tyr75	Ile85	11	15.92	YHKLIKPLNAI
5	Arg89	His104	16	23.97	RKHDFNLEKCGGQTQH
6	Val107	Glu120	14	20.38	VGKRANAYYKCLVE
7	Thr122	Ala139	18	26.99	TSGDAFKKVFDTVELVKA
8	Val151	Ile163	13	20.56	VVDKLMKKIDDKI

3.3. Structure Validation of D7r1

The structural validation and reliability of predicted model of D7r1 was carried out by several online tools such as PROCHECK, ERRAT, Verify-3D and RAMPAGE. A Ramachandran plot was built by RAMPAGE describes that 96.9% (158 aa) of residues fall in favoured region, 3.1% (5 aa) of residues fall into the allowed region and none of the residues fall in the disallowed region which reveals the precision of dihedral angles and backbone in three dimensional space (Figure 4). In addition, the quality of modeled structure was evaluated based on the energetic and empirical methods by Verify-3D, which produces average data points for each residue. In this case, a Verify-3D score of D7r1 protein model was 70.91%. ERRAT algorithm validates the reliability of the modeled structure with an ERRAT score of 92.10. In addition, PROSA web server was used to check the quality of the model in terms of Z score, a measure of overall model quality and confirms whether the target protein was within the acceptable range of X-ray and NMR studies. The Z score for the D7r1 protein model was found to be -5.61 which suggest a better quality model (Figure 5). The Q mean server was used to examine the model quality by comparing all available reference structures which are experimentally solved. This server result in six structural parameters including C_{beta} interaction energy -43.85 (Z-score: -1.77), all-atom pairwise energy -3482.66 (Z-score:-1.29), solvation energy -2.52 (Z-score:-1.88), torsion angle energy -24.36 (Z-score: -1.65), secondary structure agreement 75.8% (Z-score: -1.03), and solvent accessibility agreement 77.6% (Z-score: -0.15), which further signifies a model of better quality. The quality of computationally modeled D7r1 structure was further validated by PROSESS web server. The PROSESS score for covalent bond quality (6.5), non-covalent/packing quality (6.5) and torsional quality (6.5) prove that the predicted D7r1 model in high quality. Figure 6A represents the overall quality factor and Figure 6B shows the number of parameters with outlier for each residue.

Figure 4. Ramachandran plot of the modeled D7r1 built by RAMPAGE

Figure 5. Validation of D7r1 model by Z-score of ProSA

(A) Global Structure Assessment:

Figure 6. Quantitative evaluation of D7r1 by PROSESS server. (A) Overall quality factor (B) Number of parameters with outlier for each residue.

3.4. Helical Nature of D7r1

The phi (Φ) and psi (Ψ) values obtained from PROSESS web server is used as a measure to evaluate the helical nature of D7r1. For each helix ($\alpha 1$ to $\alpha 8$), the phi (Φ) and psi (Ψ) values are retrieved from the residual report of PROSESS web server. The backbone dihedral angles Φ and Ψ fall in a narrow region of the Ramachandran plot, with Φ between -95° and -35° , and Ψ between -15° and -70° [50]. The sum of Φ and Ψ angles on adjacent residues decides the helical form of protein, in which the angle for α -helices is approximately -105° . The sum of above mentioned angle for π -helix and 3_{10} -helix is -75° and -130° respectively [51]. The average sum values of Φ and Ψ angles for each alpha helices such as $\alpha 1$ (-99.97°), $\alpha 2$ (-98.25°), $\alpha 3$ (-103.52°), $\alpha 4$ (-108.67°), $\alpha 5$ (-103.74°), $\alpha 6$ (-104.79°), $\alpha 7$ (-102.33°) and $\alpha 8$ (-106.07°) were calculated which clearly proves the alpha helical nature of D7r1 protein.

3.5. MD Simulation of D7r1

In general, MD simulation has been used to refine and understand the stability of structure predicted by homology modeling approach [52]. Herein, the assessment of the stability of the modeled D7r1 was carried out by equilibrating the protein for a time scale of 50ns. The root-mean-square deviation(RMSD) as a standard to evaluate the structural stability of the protein for the protein

backbone atoms. The backbone RMSD profile shows the D7r1 attains equilibrium after the 15,000 ps and remains stable for rest of the simulation period (Fig.7A). Meanwhile, the root mean square fluctuation (RMSF) of D7r1 was generated to distinguish the mobility of each residue and depicted in Fig.7B. The RMSF fluctuation of D7r1 shows that it has two flexible regions (F1 and F2). The residues present in N-terminal loop region (A12-V17) shows gradual fluctuation with a value of 0.39 nm and are recognized as an flexible region (F1) of D7r1. Whereas, the high flexible region (F2) was observed in residues between K140-Q147 with an RMS fluctuation of 0.47 nm. The compactness of the D7r1 was ensured by calculating the Radius of gyration (R_g) and shown in Fig.7C. The potential energy graph in Fig.7D shows that trajectory with minimum potential energy conformation was observed at 15140 ps. Further, the minimum and maximum potential energy of D7r1 was calculated as -768433.31 and -761988.31 respectively, which explains that the predicted model were stable in terms of energetic behavior during the MD simulation. Accordingly, the minimized representative structure was retrieved from equilibrated part of trajectory by `g_dump` command in GROMACS suite. The minimized D7r1 model was subjected to structural validation by the same above mentioned tools.

Figure 7. The results of molecular dynamics simulation. (A) Backbone RMSD of D7r1 during 50ns. (B) Backbone RMS fluctuation of D7r1 depicting two flexible regions. (C) Showing Radius of gyration (Rg) of D7r1 (D) Showing Potential energy of D7r1

3.6. Principal Component and FEL Analysis of D7r1

In order to understand the structural and conformational stability of D7r1, the principal component analysis and free energy landscape analysis were performed. The first step involves generation of covariance matrix of protein alpha carbon (C α), in which 1659 protein elements were used with sum of eigenvalues to be 5.991 nm². The top eigenvectors responsible for approximately 90% of the internal motions of the protein and therefore the first five, tenth and the twentieth projections from the trajectory were retrieved and projected onto the eigenvectors against time during the 50ns simulation period (Fig.8). Further FEL plot was generated based on the cosine content values (<1) of principal components (PCs), as they are found to be capturing the functional behavior of the protein and an indicator of good simulation sampling. In our study, the FEL plot was generated for D7r1 trajectory using principal components (PC1 and PC2) to observe the conformational changes during the course of the simulation. The FEL plot shows three different energy clusters, which corresponds to different conformations of D7r1 protein. The energy cluster

C1, C2 and C3 represent the minimized structures of D7r1 at 18ns, 32ns and 40ns respectively (Fig.9A). The FEL contour map in Fig.9B also used to visualize the representative structure with minimal energy. In addition, the minimized structures from FEL analysis were retrieved and superimposed with initial predicted structure, which shows the RMSD deviation of 3.462 Å, 3.380 Å and 2.956 Å for 18ns, 32ns and 40ns representative structures respectively. Further, low energy clusters were subjected to structural validation by the RAMPAGE and the Ramachandran plot given in Fig.10. The Ramachandran plot built by RAMPAGE shows that residues falling in most favored regions found to be 86.4%, 84% and 84%, for 18ns, 32ns and 40ns respectively. Similarly, residues falling in disallowed regions found to be 3.1%, 3.1% and 2.5% for 18ns, 32ns and 40ns respectively, meanwhile the ERRAT values calculated as 94.94, 93.63 and 88.81 respectively. These validation results provide insights into the reliability of modeled structure. Further, in view of ERRAT and Ramachandran plot, 18ns refined structure considered as a good quality structure.

Figure 8. Principal component analysis (PCA) of D7r1. The motions along with the first five, tenth and twentieth eigenvectors with their respective color code during the 50ns simulation.

3.7. Active Site Prediction

The active site of D7r1 as predicted from both computational and literature studies. The COACH server, a *meta*-server, which is combined of several online servers which includes COFACTOR, ConCavity and S-SITE were used to predict the residues which are involved in the binding pocket formation (Table.3). Further, the sequence homology and structure conservancy between D7r1 and template 2PQL shows high degree of consistent [10]. Hence, the active site residues of *Anopheles gambiae* D7r4 and ligand tryptamine ($C_{10}H_{12}N_2$) were retrieved by submitting 2PQL to PDB sum [53] server. Clustal Omega [20] tool used to align the

sequence of the target and template protein and the corresponding active site residues were predicted. The amino acid residues Ile42, Arg43, Tyr45, His56, Met57, Val60, Phe131, and Met156 are identified as active site residues.

Figure 9. PCA based Free energy landscape (FEL). (A) FEL representative energy cluster are shown as C1, C2 and C3 for 18ns, 32ns and 40ns respectively. Corresponding representative structure are retrieved and displayed accordingly. (B) FEL contour map which depicts lowest energy conformation with their respective structure.

Table 3. Predicted active site residues by COACH, COFACTOR, ConCavity and S-SITE server with their respective C-score.

Server Name	Ligand name	Residue Numbers	C-score
COACH	SRO	V24, E28, I42, R43, Y45, H56, M57, V60, Y115, F131, D132, E135, M156	0.79
COFACTOR	HSM	I42, R43, Y45, Y115, F131, D132, E135	0.56
ConCavity	Cavity 1	V24, E28, C40, I42, R43, K44, Y45, H56, M57, V60, Y115, F131, D132, E135, D160	0.23
S-SITE	SRO	V24, E28, I42, R43, Y45, H56, M57, V60, Y115, F131, D132, E135, M156, D160	0.48

3.8. Disordered Region Prediction and Evolutionary Conservation Analysis

In proteomics, the disorder can be defined as the lack of regular secondary structural elements and high degree of flexibility in the protein structure. The high flexibility of the disordered region in a protein might help to adopt different conformational possibilities. Further, the disordered region contains functional sites or linear motifs which influence the functionality of the protein. Therefore, to identify globular and disorder regions in D7r1 protein sequence GlobPlot were used and it is anticipated that two regions 69-78 and 100-105 are intrinsically disordered within the protein sequence. In X-axis, the blue color coded regions are disordered regions and green colored regions are ordered or globular regions of D7r1 (Fig. 11).

Figure 10. Comparative diagram representing Ramachandran plot of D7r1 of Pre and after MD simulations at 18ns, 32ns and 40ns. The plots were built by RAMPAGE

Figure 11. The globular and disordered regions of D7r1 shown in green and blue color respectively

4. Conclusions

Owing to the importance of the saliva protein D7r1 of *Anopheles gambiae* as an anti-malarial target, the present study reports the *in silico* structural analysis that providing insights into its structural architecture. *In silico* analysis has reached to such advancement that results obtained from computational study are much more reliable and comparable to experimental results. The three dimensional structure of D7r1 has been predicted through homology modeling which shows structural similarity to its experimentally determined template structure (PDB ID: 2PQL). Similarly, the predicted D7r1 also contains eight helices connected by several loops which resemble the structural architecture of D7r4. At present, the molecular dynamics simulation study proven to be an essential step in computational biology which provides insight into protein structural stability through RMSD and RMSF plot. In our study, to explore the conformational flexibility and structural transition occur in D7r1, essential dynamics were employed which shows that three cluster basin at different time interval. Accordingly, FEL representative structures at different conformational state were retrieved and validated through Ramachandran plot. Further, similar to D7r4, same active site residues involved in binding pocket formation and opened a gateway to investigate the interacting mechanism of D7r1 with its suitable ligands and protein interacting partners through molecular docking study. Since, the structural knowledge of macromolecule is an important pre-requisite to understand its functional role. Hence, the predicted D7r1 structure and its active site might be useful in further attempts to elucidate the experimental structures as well as to design new lead molecules acting on novel drug targets considered as a suitable starting point for structure based drug design in the treatment of malaria.

Conflicts of Interests

Authors declare no conflicts of interest.

Author Contribution

Manikandan Jayaraman and Chayanika Goswami contributed equally.

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