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Insilico modeling of *Wolbachia* and its potentials in combating mosquito borne diseases Chikungunya and Dengue

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ABSTRACT
Mosquito borne diseases are major health burden both in tropical and subtropical regions**.** The enormous use of insecticides to control mosquitoes causes biomagnification of chemicals in environment and mosquitoes have developed resistance to insecticides. The inefficiency of insecticides to combat mosquitoes prompted researchers to develop efficient alternative methods. *Wolbachia* endosymbiont is a one of efficient new approach to control mosquitoes. *Wolbachia* strain invade mosquitoes biology by reducing host lifespan, phenotype and inhibit virus replication. In the present study, *insilico* modeling and docking of *Wolbachia* and human pathogens Chikungunya (CHIK) and Dengue (DEN) virus was done. Docking is the method to find the binding affinity of protein and ligand complex molecules for finding potential inhibitor. Using Hex, we obtained energy total (e-total) values in kcal/mol for all docked complex. In the contest of overall analyzing the docking E-total values of docked complexes reveals that WSP-B has show strong binding affinity than WSP-A to both DEN and CHIK. Based on obtained result, we suggest WSP-B has potential inhibitor for both DEN and CHIK virus. Further, biophysical characterization of *Wolbachia* will help to develop a drug to combat CHIK and DEN viruses.

Keywords: *Wolbachia*, Virus, Mosquito, models, Disease and Control.

1. Introduction
Arthropod borne diseases, are among the leading cause for health burden in humans. Mosquito-borne diseases such as malaria, CHIK, DEN fever, yellow fever, West Nile virus, Japanese encephalitis and lymphatic filariasis cause an enormous health burden to people living in tropical and subtropical regions of the world. Estimates made by the World Health Organization (WHO) show that 247 million people become ill in 2006 and about one million people died from mosquito borne diseases [1]. The use of insecticides to target mosquitoes as a means of disease control can be effective, but is often prohibitively expensive, unsustainable and environmentally undesirable. Furthermore, repeated exposure of mosquitoes to insecticides has allowed insecticide resistance to develop, increasing the need to use more expensive alternative compounds. Despite years of intense effort to control them, many of these diseases are increasing in prevalence, geographical distribution and severity. The options to control them are limited [2].
The symbiotic bacteria *Wolbachia pipientis* is a maternally transmitted intracellular bacteria most common and widely spread in insects, including mosquitoes [3, 4, 5]. *Wolbachia* manipulates host biology in many ways, including the cytoplasmic incompatibility (CI) whereby mechanism referred to incompatibility between sperm and egg it results in embryonic mortality in *Wolbachia* infected males crosses with uninfected female or is infecting with different strain of *Wolbachia*. During the 1967 *Wolbachia* induced CI was proposed to control *Culex* mosquitoes [6]. This technique was used in India to combat mosquitoes during 1970s [7]. In recent years resurgence of *Wolbachia* potential have been explored to control mosquito borne diseases such as malaria, DEN and CHIK [8].
In the present study, *Wolbachia* strains WSP-A and WSP-B of uzifly, *Exorista sorbillans* is used to know the interaction of *Wolbachia* against CHIK and DEN viruses. The proposed study highlights use of *E. sorbillans Wolbachia* strains that will be characterized for future evaluation of their efficacy to combat arboviruses CHIK and DEN.

2. Material and Method

Insilico study of molecules were carried out using several online tools NCBI (http://www.ncbi.nlm.nih.gov/), EMBL (www.ebi.ac.uk/), ITASSER [9], Q-site (http://www.modelling.leeds.ac.uk/qsitefinder/) finder and software program Hex [10] Rampage (http://mordred.bioc.cam.ac.uk) and PyMol (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC). The DNA sequence of DEN, CHIK, WSP-A and WSP-B were retrieved from the NCBI database. Retrieved DNA sequence was converted to protein sequence by using EMBL protein converter program EMBOSS Transeq. The converted proteins were submitted to ITASSER homology modeling server to obtain 3D structure. The modeled structures were validated using RAMPAGE program. MGL tools [11] used to add hydrogen molecules. Using Hex we modeled complex of DEN;WSP-A, DEN:WSP-B, CHIK:WSP-A, CHIK:WSP-B and evaluated binding affinity of complex molecules by obtaining docking energy.

2.1 Sequence retrieval

The DNA sequence of WSP sequences of *Wolbachia* of *Exorista sorbillans* sequences WSP-A-JN102345, WSP-B-JN102346 and DEN-L11422.1 and CHIK-EU856108 were retrieved from the NCBI. All the sequence was subjected to translating in to protein sequence.

2.2 Translating DNA sequence to protein

Due to lack of protein sequence of WSP-A, WSP-B, DEN, CHIK, we used corresponding DNA sequence to get protein sequence to respective model protein structure. Here obtained DNA sequence subject to translate to protein sequence by using EMBL server. EMBOSE transeq algorithm used to perform DNA to RNA translation. EMBOSS transeq translate nucleic acid sequence to their corresponding protein sequence (http://www.ebi.ac.uk/Tools/st/emboss_transeq).

2.3 Protein structure prediction

2.3.1 Modeling

Model building server I-TASSER used to predict the 3D structure of proteins. Translated protein sequences were submitted to I-TASSER. Protein Templates selected based on Template Modeling Score (TM-score-is an algorithm used to calculate the likeness of topologies of two protein structures) used to predict 3D structure of query sequence. Based on these templates I-TASSER predicted five models computationally by using c-score algorithm value -5 to 2, c-score represent the confidence score of model and if c-score increases, confidence of the model too increases. We chosen best model among the 5 suggested models based on c-score.

Validation of homology modeling

Modeled 3D structure of WSP-A, WSP-B, CHIK and DEN were subjected to its quality checked through RAMPAGE for bond angel. The Ramachandran plot by Rampage provided the residue position in particular segment based on the dihedral angles. It also generate amino acids plot for allowed and disallowed region [12].

2.3.2 Receptor preparation

DEN and CHIK protein are used as receptor for our study. After modeling these proteins, we subjected to check the stereochemical stability and added missing hydrogens using MGL tools.

2.3.3 Ligand preparation

WSP-A and WSP-B modeled proteins used as ligand molecules in protein-protein docking. Before performing docking, checked bond orders, missing hydrogen bond and stereochemical structure and any clashes by using MGL tools.

2.3.4 Active site prediction

Molded proteins of DEN and CHIK were submitted to Q-site finder for analysis of active site. Q-Site Finder is online program used for ligand binding site prediction. Q-site finder performs by binding hydrophobic (CH3) probes to the protein, and finding clusters of probes with the most favorable binding energy. The clusters are used in to keep in rank order of most potential binding site by calculating each clusters sum total binding energies [13]. Results obtained by Q-site finder analyzed thoroughly for probability of existing active site and amino acids which are essential for indigenous reactive group involved in interaction with other ligand molecules.

2.3.5 Docking

Docking was performed with Hex 6.12. Hex is protein-protein docking software developed to analyze binding affinity and molecular interactions. Protein-ligand docking models are also can develop using this Hex [14]. Docking is the method to predict the enzyme-ligand complex interaction in binding mode. Parameters used in Hex docking protocol for analyze lowest E-total evaluation is based on correlation type is “shape only”. First Fourier transform (FFT) mode is used in our docking process is 3D and adjusted grid dimension is 0.75, and remaining all parameters is used as default. Docking process develop lowest energy clusters for possible conformers at every interaction site. Final interaction of ligand analyzed based on cluster conformation formed at active site and involved in hydrogen bond formation with amino acids located at active site and highest ranking score build at interaction of protein and protein. Docking results generated 500 lowest energy cluster confirmation models for every docking complex. Post Processing applied 1000 solution to carry out filter and refine the confirmed clusters. Steric clashing clusters are removed in post process by using Clustering control panel by bump counter. Post processing applied to minimize each docking solution by calculating molecular mechanic energy or Newton like energy by using ‘soft’ Lennard-Jones and hydrogen bond potential implemented from the OPLS force field parameters. Root Mean Squares Deviation (RMSD) value -1 is used. Including this mainly good docking solution is selected based on lowest docked E-total [15]. For this study we used Intel core i5 processor, 4GB ram desktop system.

3. Results and Discussion

Proteins sequences of WSP-A, WSP-B, DEN and CHIK were obtained by using several online bioinformatics tools and used for further studies.

3.1 Homology modeling.

Protein sequence submitted into I-TASSER server for modeling 3D structures of proteins WSP-A, WSP-B, DEN and CHIK. The predicted 3D structures were than subjected to docking studies for further analysis. We selected most favorable model for further analysis among 5 structures suggested by I-TASSER. Modeled all four structures are shown in Figure-1 which were selected for docking simulation analysis for binding affinity and molecular interaction between them.

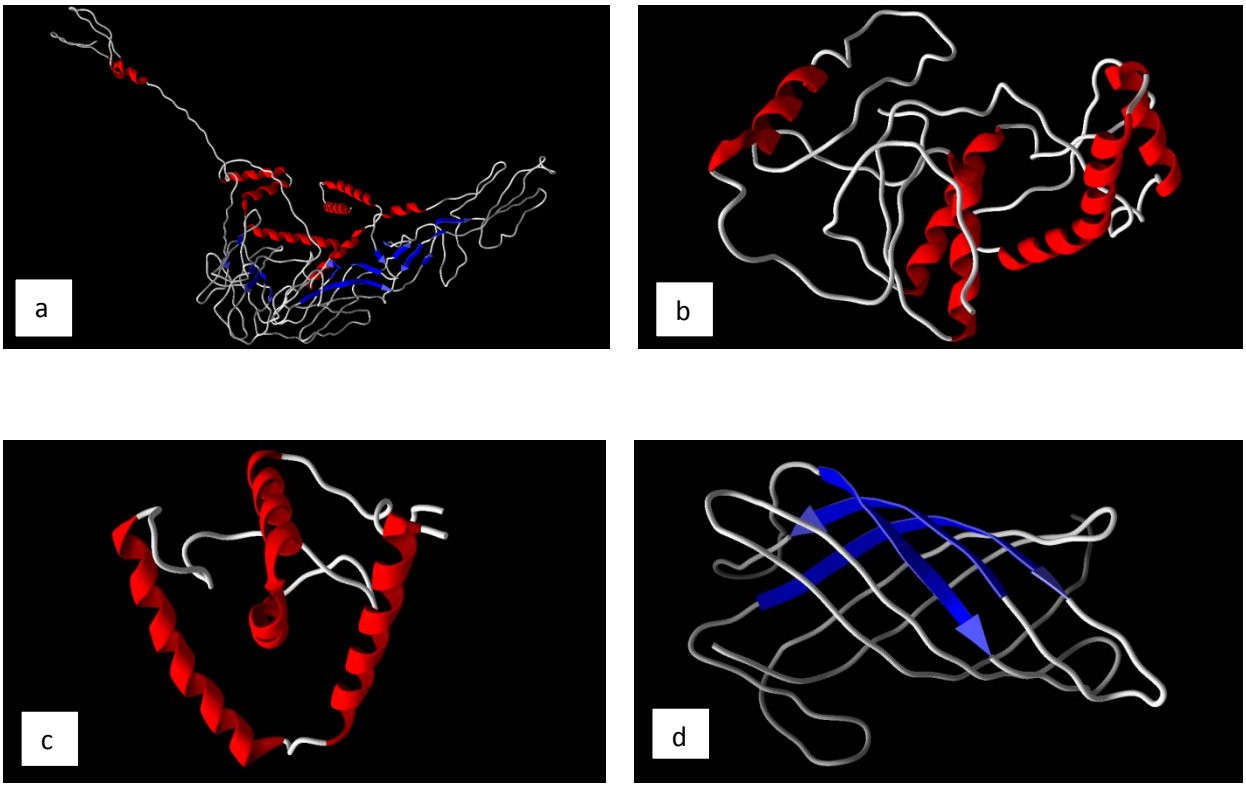
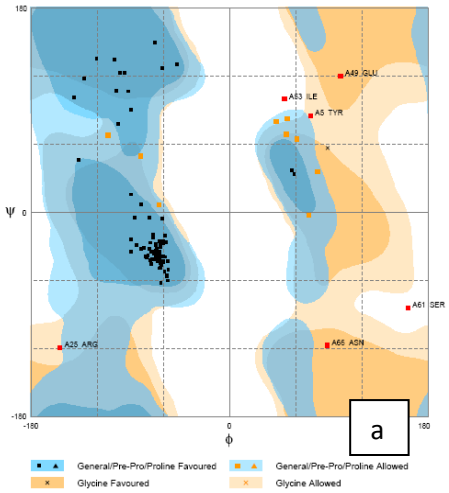
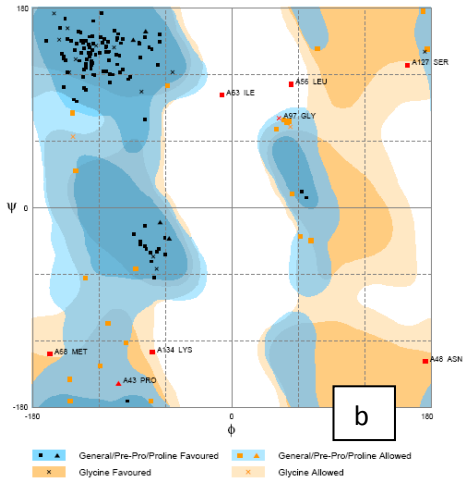


Fig 1: Modeled structure of back bone visualization of a) DEN, b) CHIK, c) WSP-A and d) WSP-B protein by using I-TASSER.

Validating homology modeling

A good quality Ramachandran plot has over 90% in the most favored regions [16] but the Ramachandran plot of WSP-A and DEN respectively has only 86.8% and 88.0% of residues in the most favored region respectively showed in table 1. Therefore WSP-A and DEN are a near to good quality model shown in Figure-2(a) and Figure-2(d). Similarly, the Ramachandran plot of WSP-B and CHIK shown in Figure-2(b) and Figure-2(c), respectively, has 79.6% and 79.0% residues in the most favored regions.





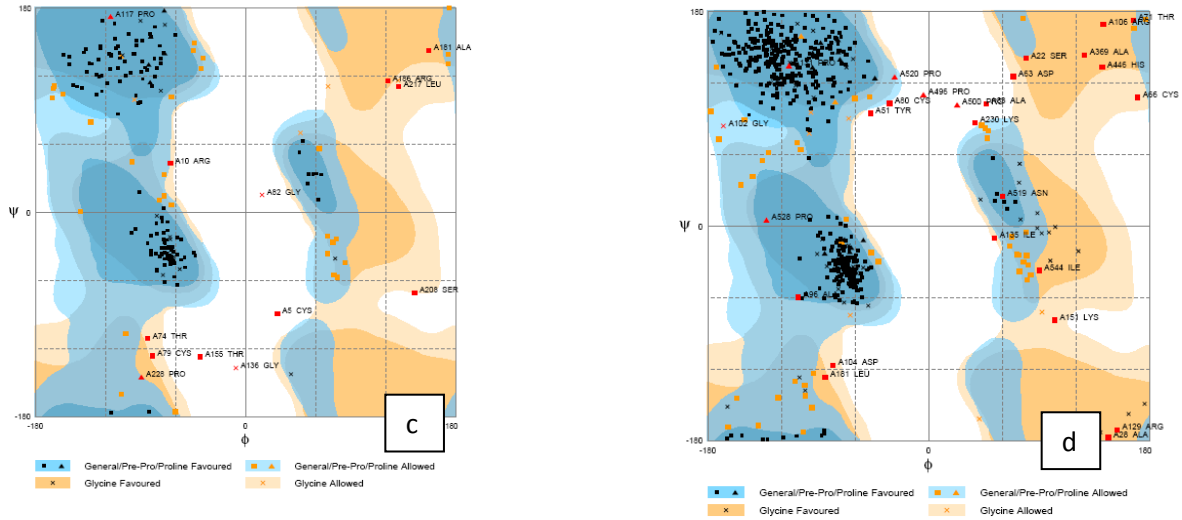


Fig 2: Analysis of modeled proteins of a) WSP-A, b) WSP-B, c) CHIK and d) DEN by RAMPAGE

Table 1: show list of amino acids present in favored and allowed region include outlier region.

SN		WSP-A	WSP-B	CHICK	DEN
1	No. of residues in favored region (~98.0% expected)	99 (86.8%)	121 (79.6%)	181 (79.0%)	577 (88.0%)
2	No. of residues in allowed region (~2.0% expected)	9(7.9%)	23 (15.1%)	35 (15.3%)	53 (8.1%)
3	No of residues in outlier region	6(5.3%)	8 (5.3%)	13 (5.7%)	26 (4.0%)

3.2 Active site prediction

Before docking any protein-protein and protein- ligand, it is good to know the binding site and residues which may involve in important role in enzymatic interaction for increasing the precise result analysis. Active site are predicted by Q site finder online tool. Q site finder uses the van der Waals prob to find energetically favored binding site. This predicted sites are clustered according to their spatial proximity and ranked them accordingly their interaction energies total [11]. This method mainly applied in the genomic studies where protein binding sites are remain uncharacterized. Using this method, Predicted active sites present in modeled proteins were shown in Figure-3.

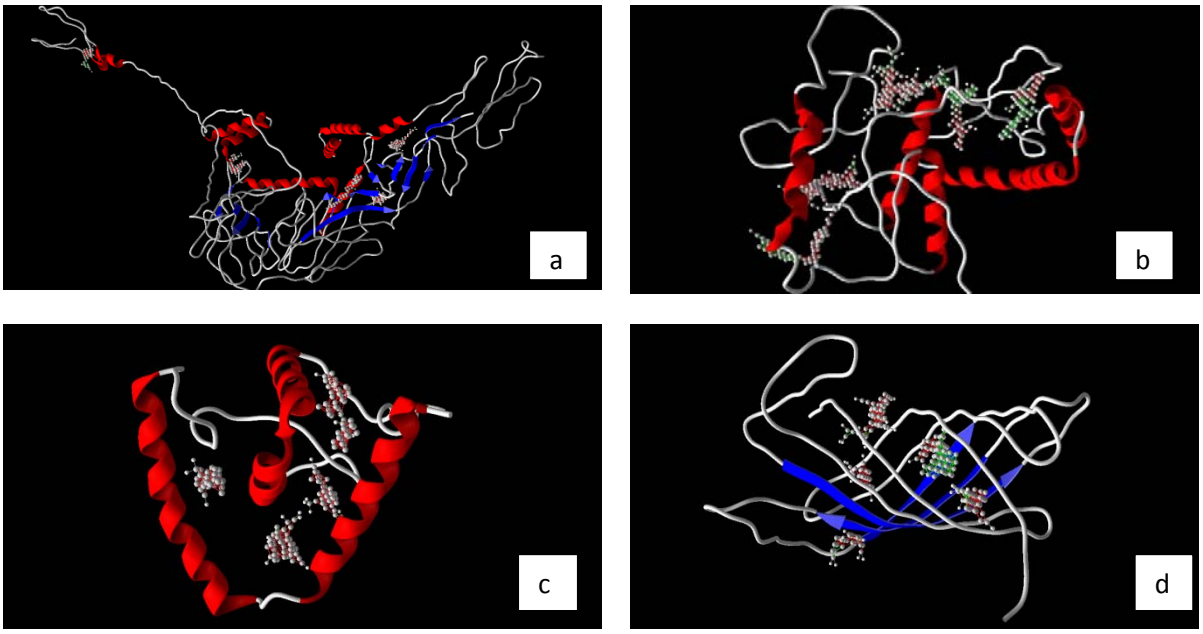


Fig 3: Active site showing as in ball and stick model in modeled structure as shown in backbone visualization of a)DEN b)CHIK c)WSP-A, and 4)WSP-B by using q site finder.

3.3 Docking

Docking of WSP-A, WSP-B with DEN and CHIK were performed and evaluated binding affinity of docked complex molecule by using the E-total value. Docked complex models were shown in Figure-4A, 4B and 5A, 5B. If E-total value decreases for docked complex, binding affinity increases E-total values show the how much stability of docking complex. E-total value for docked complex models DEN: WSP-A, DEN: WSP-B, CHIK: WSP-A and CHIK:WSP-B shown as -8.285, -9.708, -4.943, and -7.892 kcal/mol. respectively. This result analysis predicts best docking complexes which have lowest E-total values. In term of binding affinity, obtained results suggest that WSP-A has good binding affinity to DEN than to CHIK. In case of WSP-B, results shows it has more binding affinity to DEN than to CHIK. Overall analyzing the docking E-total values suggest WSP-B has shown strong binding affinity to both DEN and CHIK than WSP-A. Further the interaction of amino acids present in active site of DEN and CHIK are suggest DEN active site is overlapping for both WSP-A and WSP-B. In CHIK different site may exits and WSP-A and WSP-B bind in different amino acids which present at active sites, amino acids involved in binding interaction were listed in table 2.

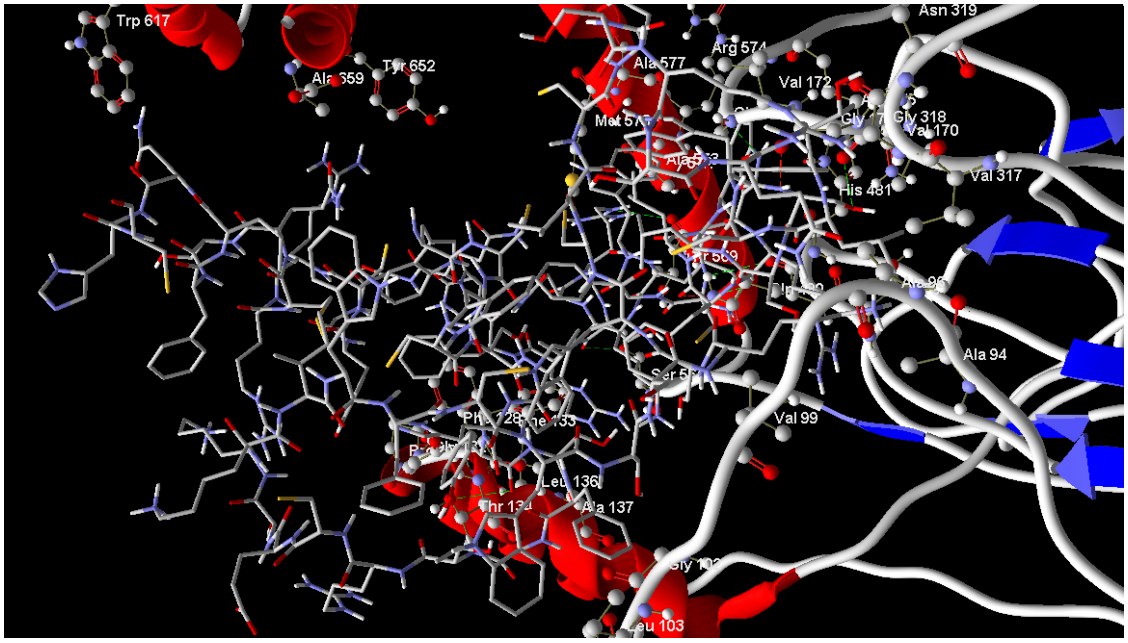


Fig 4A: Docking models of DEN:WSP-A. Interaction of Amino acid molecules present in active site of DEN showing in ball and stick model make H-bond interaction with WSP-A. WSP-A show in Stick model present at active site after docking with DEN.

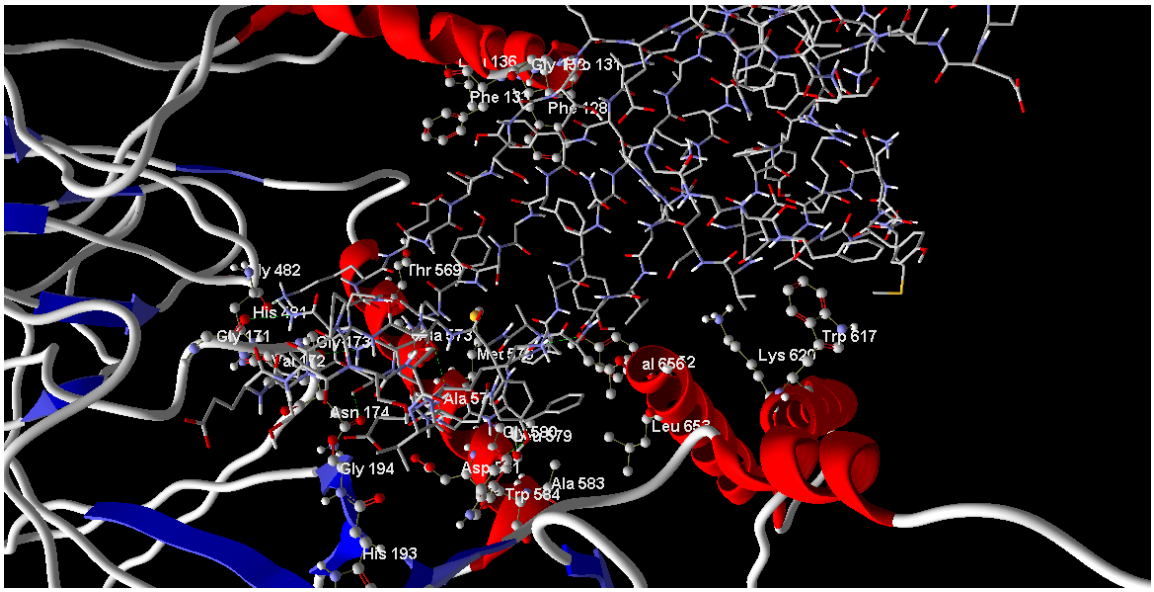


Fig 4B: Docking models of DEN:WSP-B. Interaction of amino acid molecules present in active site of DEN showing in ball and stick model make H-bond interaction with WSP-A. WSP-B show in stick model present at active site after docking with DEN.

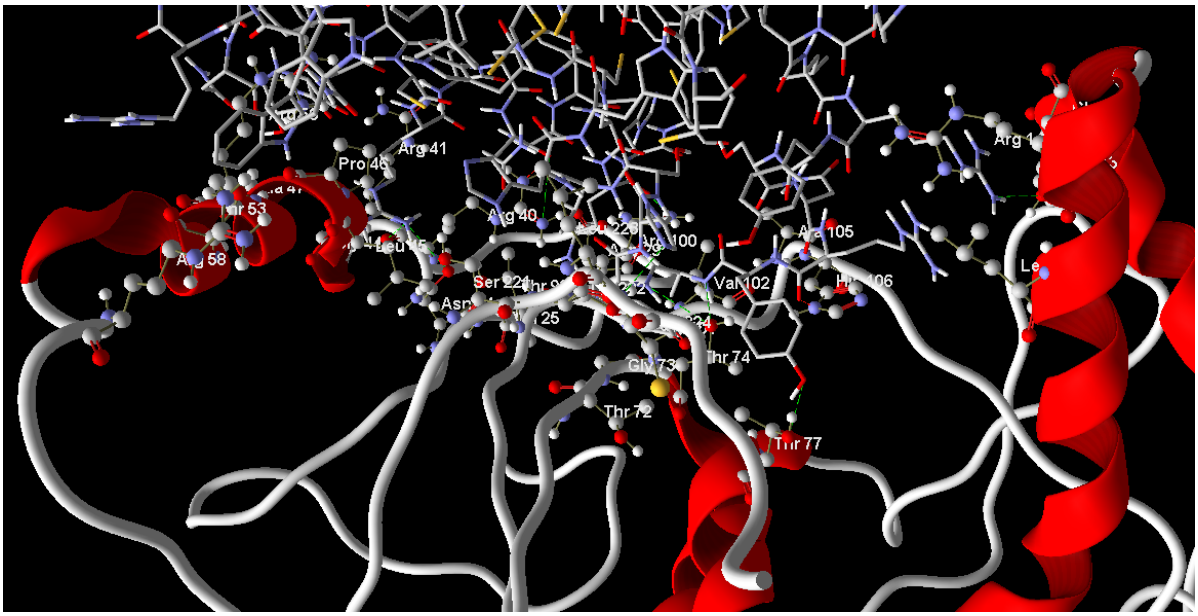


Fig 5A: Docking models of CHIK:WSP-A. Interaction of Amino acid molecules present in active site of CHIK showing in ball and stick model make H-bond interaction with WSP-A. WSP-A showing in stick model present at active site after docking with CHIK.

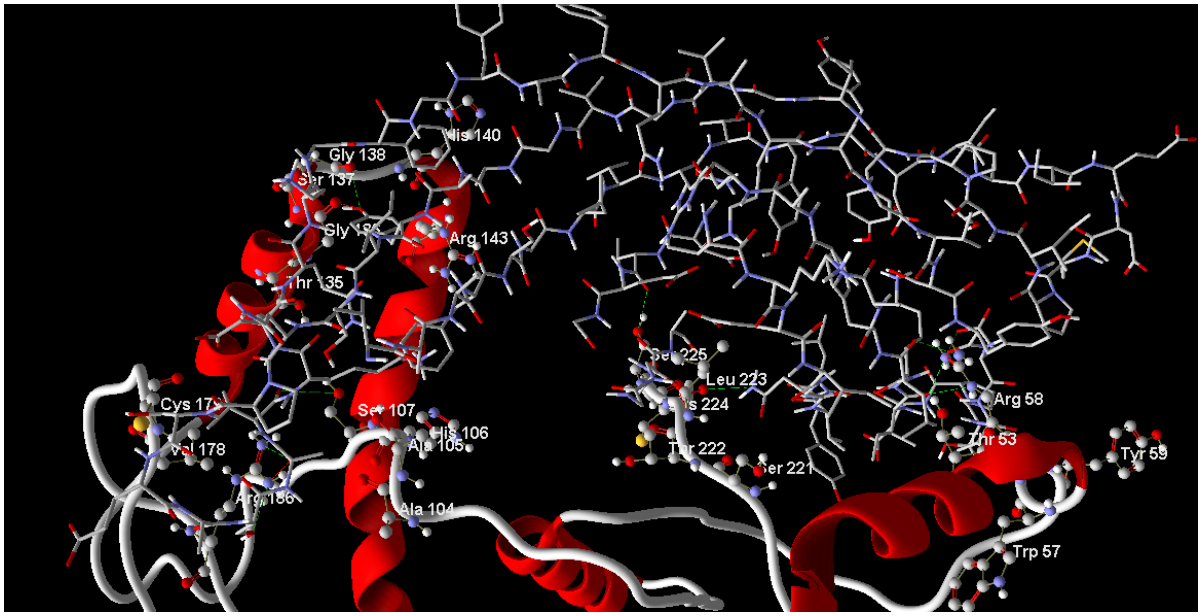


Fig 5B: Docking models of CHIK:WSP-B. Interactions of Amino acid molecules present at active site of CHIK showing in ball and stick model make H-bond interaction with WSP-B. WSP-B shows in stick model present at active site after docking with CHIK.

Table 2: Show list of amino acids present in active site of docking receptor and ligand complex.

S.N	DEN:WSP-A	DEN:WSP-B	CHIK:WSP-A	CHIK:WSP-B
1	Ala 94,Ala 96, val 99, Gly 102, Leu 103, Phe 128, Pro 131, Gly 132, Phe 133, Thr 134, Leu 136, Ala 137, Val 170, Gly 171, Val 172, Gly 173, Arg 175, val 317, Gly 318, Asn 319, Thr 479, Gln 480, His 481, Ser 561, Thr 569, Gly 572, Ala 573, Arg 574, Met 576, Ala 577, Trp 617, Tyr 652, Ala 659	Phe 128, Pro 131, Gly 132, Phe 133, Leu 136, Gly 171, Val 172, Gly 173, Asn 174, His 193, Gly 194, His 481, Gly 482, Thr 569, Ala 573, Met 576, Ala 577, Leu 579, Gly 580, Asp 581, Ala 583, Trp 584, Trp 617, Lys 620, Tyr 652, Leu 653, Val 656	AS24, Val 25, Asp 29, Arg 40, Arg 41, Gly 44, Leu 45, Pro 46, Ala 47, Arg 50, Thr 53, Arg 58, Thr 72, Gly 73, Thr 74, Thr 77, Thr 99, Arg 100, Gly 101, Val 102, Ala 105, His 106, Thr 135, Gly 136, Arg 143, Leu 146, Ser 222, Leu 223, Cys 224	Thr 53, Trp 57, Arg 58, Tyr 59, Ala 104, Ala 105, His 106, Ser 107, Thr 135, Gly 136, Ser 137, Gly 138, His 140, Arg 143, Val 178, cys 179, Arg 186, Ser 221, Thr 222, Leu 223, Cys 224, Ser 225

4. Discussion

The ability of *Wolbachia* to inhibit or block DEN and CHIK proliferation in a hosts makes it a potential "mosquito vaccine" that could be used efficiently to prevent pathogen transmission [17, 18, 19]. Further, *insilco* and *in vitro* studies of *Wolbachia*, DEN and CHIK can require to know the active pathways. More detailed understanding of molecular mechanisms of *Wolbachia* effects on DEN and CHIK virus is importance for refining utilization of *Wolbachia* system for the control of DEN and CHIK.

Docking is the method to find the binding affinity of protein and ligand molecules for finding potential inhibitor. Using docking studies, we have revealed the mechanism and mode of action of protein-protein interaction and binding affinity. By using E-total concept, we found the potential inhibitor protein molecules by analyzing docking results. In the contest of overall analyzing the docking E-total values in term of binding affinity suggests WSP-B has shown strong binding affinity than WSP-A to both DEN and CHIK. Docking results suggest use of WSP-B as potential inhibitor for both DEN and CHIK causing diseases. For effective inhibition activity, obtained results show WSP-A used as potential inhibitor for DEN and WSP-B can be used as inhibitor for both the CHIK and DEN.

In the present study, *insilico* modeling shows *Wolbachia* strains WSP-A and WSP-B have the different varying abilities to inhibit DEN and CHIK pathogens. This may be perhaps a promising implication *Wolbachia* –mediate the protection of viruses [20, 21]. Modeling studies on different pathways like Toll, Imd and JAK-STAT will yield exact mechanisms immune signaling of *Wolbachia* to combat DEN and CHIK pathogens is of obvious concern [22]. Further, *Wolbachia* cell culture and host (Mosquito) interaction studies on small RNA breakdown pathways will yield efficient mechanisms to control above said pathogens [23, 24].

5. Conclusion

The *Wolbachia* and pathogen interactions are very important in understanding the mechanisms in the process of new drug development program. Further, biophysical studies of *Wolbachia*, DEN and CHIK virus can provide the physical, chemical and functional mechanisms. The present study serves as a basis, in understanding the molecular interaction mechanisms of *Wolbachia* and pathogens is an important refining system in developing a drug to combat DEN and CHIK pathogens.

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