SRI VENKATESWARA INTERNSHIP PROGRAM
FOR RESEARCH IN ACADEMICS
(SRI-VIPRA)

SRI-VIPRA

Project Report of 2023: SVP-2336

"*In silico* Screening and Identification of Potential Inhibitors of Zika Virus Protein NS5 by structure-based virtual screening"

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SRIVIPRA PROJECT 2023

Title : "*In silico* Screening and Identification of Potential Inhibitors of Zika Virus Protein NS5 by structure-based virtual screening"

Name of Mentor: Dr. Sumit Raj **Name of Department: Zoology** **Designation: Assistant Professor**	

List of students under the SRIVIPRA Project

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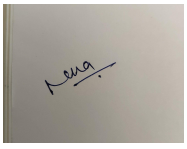
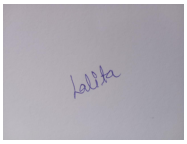
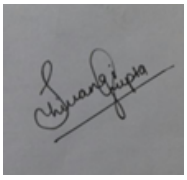
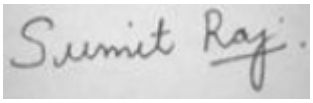
2		Neha Mahour	1121123	B.Sc.(P) Life Sciences	

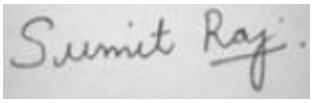
3		Lalita	1121034	B.Sc.(P) Life Sciences	
4		Shivangi Gupta	2021030	B.Sc(H) Zoology	



Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP titled **"*In silico* Screening and Identification of Potential Inhibitors of Zika Virus Protein NS5 by structure-based virtual screening"**. The participants have carried out the research project work under my guidance and supervision from 15th June 2023 to 15th September 2023. The work carried out is original and carried out in an online/offline/hybrid mode.



Signature of Mentor

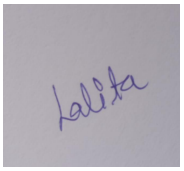
Acknowledgements

The internship opportunity we had with SRIVIPRA was a great chance for learning and professional development. Therefore, we consider ourselves very lucky individuals as we were provided with an opportunity to be a part of it. We are also grateful for having a chance to meet so many wonderful people and professionals who led me through this internship period.

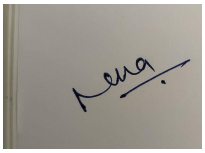
Bearing in mind the previous we are using this opportunity to express my deepest gratitude and special thanks to Dr. Sumit Raj who in spite of being busy with his duties, took time out to hear, guide and keep us on the correct path and allowed us to carry out the project at this esteemed organization and extending during the training.

We perceive this opportunity as a big milestone in our career development. We will strive to use gained skills and knowledge in the best possible way. We are extremely grateful to our college staff members and friends who helped us in the completion of this internship.

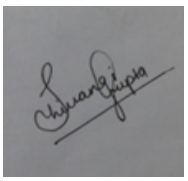
Jeevika Bhat



Lalita



Neha Mahour



Shivangi Gupta

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1.Abstract:

Zika virus (ZIKV) has emerged as major health concern, as ZIKV infection has been shown to be associated with microcephaly, severe neurological disease and possibly male sterility. As the largest protein component within the ZIKV replication complex, NS5 plays key roles in the life cycle and survival of the virus through its N-terminal methyltransferase (MTase) and C-terminal RNA- dependent RNA polymerase (Rdrp] domains. Here, we analyzed the crystal structures of ZIKV NS5 MTase in complex with an RNA cap analogue (m7 GpppA) and the free NS5 RdRp. We have identified the conserved sequences of ZIKV NS5 MTase and RdRp structures in the catalytic sites that could lead to development of current antiviral inhibitors being used against flaviviruses, including dengue virus and West Nile virus, to treat ZIKV infection. We have also identified novel potential inhibitors of ZIKV NS5. These results should inform and accelerate the structure-based design of antiviral compounds against ZIKV.

2. Introduction

Zika virus is a member of the Flaviviridae family of viruses, which also includes some other significant viruses that pose a concern worldwide, such as Dengue (DENV), Japanese encephalitis (JEV), Yellow fever (YFV), and Tick-borne- diseases. **(Song et al., 2021)** Zika virus was initially believed to be exclusively transmitted by the *Aedes* mosquito, however, research conducted in 2016 has shown that congenital, perinatal, and sexual transmission also occurs. **(Ramharack & Soliman, 2018)** Zika virus infection during pregnancy could result in major birth defects and is linked to certain other pregnancy issues. It may also cause some serious diseases that affect the brain, swelling of the brain or spinal cord, or a blood abnormality, it may lead to infertility in males and hence this infection proves itself to be a global hazard. There are currently no known vaccinations or medications that can effectively prevent or treat ZIKV infection; therefore, to control the cases of infection, the development of anti- ZIKV agents is urgently needed. **(Shi & Gao, 2017)** The development of powerful inhibitors against the virus that not only totally eradicate the infection but also mask it requires a rapid and pragmatic drug formulation. The quick screening of new prospects is made possible through in silico drug design, which reduces the necessity for time and resources. Theaflavin, a polyphenol derived from tea is shown to inhibit the ZIKV MTase activity by a concentration of 50%. **(Ramharack & Soliman, 2018)**

ZIKV is a single-stranded positive-sense RNA virus with a small envelope. This polyprotein is encoded by its genome and is made up of 3 structural proteins- the capsid (C), membrane (M), and envelope (E) and 7 non-structural proteins. NS5 is one among the non-structural proteins and is further sub-divided into three domains, i.e., N-terminal methyltransferase (MTase) domain, a C-terminal RNA- dependent RNA polymerase (RdRp) domain, and an inter-domain with residues that connects the MTases and RdRp domains. **(Zhang et al., 2017)** The MTase domain is crucial for therapeutic research as the enzyme nucleoside 2'O and N-7 methylases the viral RNA cap, which is critical for the virus's ability to replicate. S-Adenosyl Homocysteine (SAH) is released from the MTase domain once the methylation process is completed. It will be hazardous for the ZIKV to proceed further if MTase is inhibited. The beginning of RNA synthesis, which results in both plus and minus-strand RNAs, is enabled by the conserved RdRp domain. Since the RdRp enzymes and their analogues are not present in the human body, inhibitors may not have any serious and harmful consequences, making them an ideal target for therapeutic research. **(Ramharack & Soliman, 2018)**

2. Literature Review-

2.1 Structure of the protein:

The protein structure consists of two chains- the methyl donor (S-adenosyl-L-methionine [SAM]) is attached to one chain, which has a lot of structural similarities to the dengue virus (DENV) MTase. The second chain lacks SAM and exhibits alpha-helix conformational changes that aid in SAM and RNA binding. A sulfate that is close to glycerol is accommodated by the SAM binding site in the second chain, which might be used as a starting point for structure-based drug formulation.

The 11 kb positive-sense RNA and 5' cap structure that make up the ZIKV genome are translated into long open polyprotein by the multi-protein replication complex (RC). This polyprotein is then broken down into three structural proteins (capsid, PrM, and envelope) and seven non-structural (NS) proteins by both NS proteins and host cofactors (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The processing of viral polyproteins and RNA synthesis, such as RNA maturation, splicing, and nuclear export, is reported to include coordinated action of the NS protein. Non-structural protein 5 (NS5), which is linked to the generation of new virus particles, was identified as the biggest and most conserved domain of the RC through the structural characterization of ZIKV proteins. As a result, it has been suggested as a possible target for therapeutic development against ZIKV.**(Bharadwaj et al., 2021)**

An RNA- dependent RNA polymerase (RdRp) domain at the C-terminal and a methyltransferase (MTase) domain at the N-terminal make up the two domains of the Zika NS5 that complement one another's abilities. NS5 protein is critical for viral replication, survival, and immune system evasion.**(Elshahawi et al., 2019)**

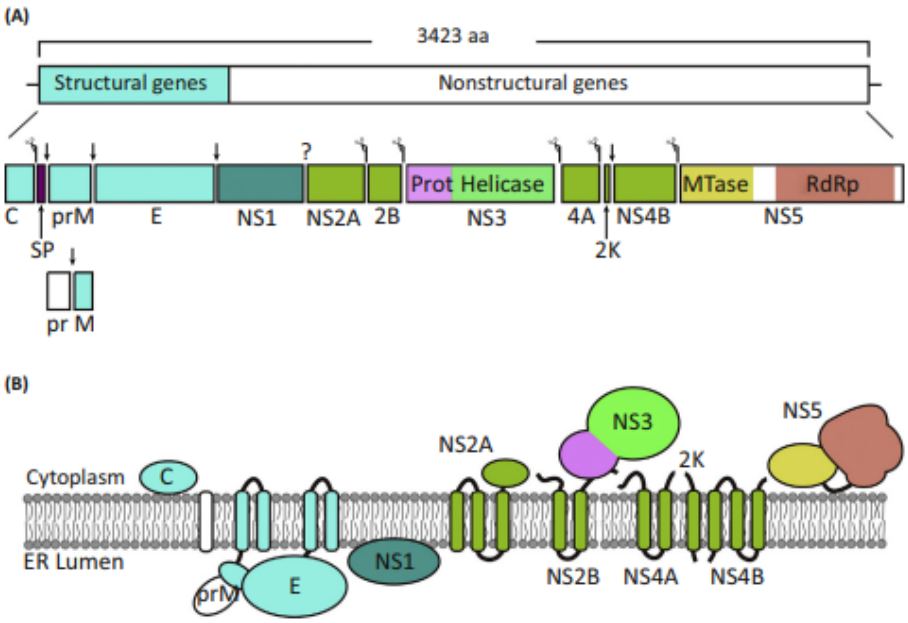


Figure 1- (a) Representing the structural and non-structural polyproteins. **(b)** Representing the topology of the polyproteins in the membrane.**(Shi & Gao, 2017)**

Three domains have been identified in the NS5 protein:

(i) N-terminal methyltransferase (MTase) domain which is essential for capping viral RNA.

(ii) a C-terminal RNA- dependent RNA polymerase (RdRp) domain, which contains the traditional thumb, palm, and fingers domains found in all single-subunit polymerases and is responsible for the synthesis of viral RNA.

(iii) an inter-domain that connects the MTases and RdRp domains. It comprises an α- helix and two β-strands. Although RdRp is necessary for all RNA viruses and MTases are frequently found in viruses with a 5' cap structure, the flavivirus NS5 is an extraordinary example of how these two critical enzymes naturally combine.

The first transcribed nucleotide is methylated at its 2'-O position to produce 7MeGpppA2'OMe-RNA, and the cap guanine is methylated at its N7-position to produce 7MeGpppA-RNA. The ZIKA MTase domain catalyzes these two methylation events. **(Zhang et al., 2017)**

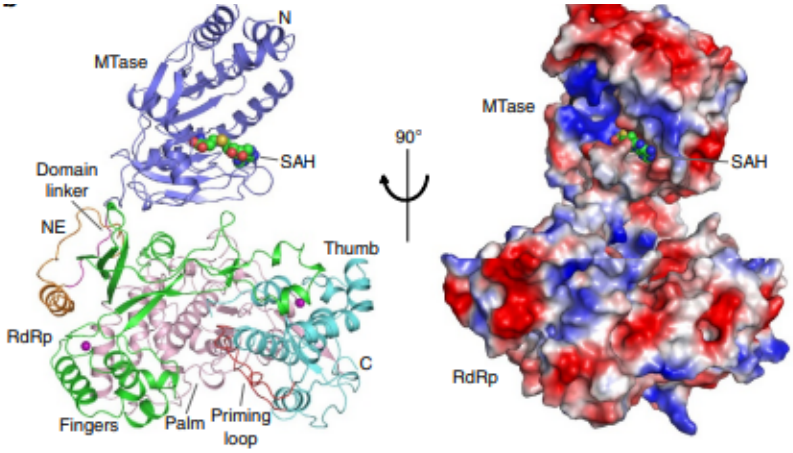


Figure 2- Representing the ribbon and electrostatic surface structure of ZIKV NS5 **(Wang et al., 2017).**

2.2 Functions of the protein-

The largest ZIKV non-structural protein, known as NS5, is involved in host interferon response suppression, RNA capping, and viral genome replication. It might also have an impact on how cellular splicosome activity is regulated. The viral RNA must be capped for the virus to remain stable and translate viral polyproteins more effectively.

It works separately with RdRp and the methyltransferase domain to replicate the viral DNA. ZIKV MTase has the ability to modify RNA cap structures by methylating them at N-7 position of the cap and at 2'-O position on the ribose of the first nucleotide, resulting in a cap-1 structure. In order to methylate the cap at the 2nd OH of the RNA, NS5 MTase first forms an enzyme-substrate complex with a GMP molecule. This is followed by the transfer of the GMP to the 50 diphosphate end of the RNA using S-Adenosyl Methionine (SAM) as a methyl donor.**(Elshahawi et al., 2019)**

MTase, a crucial component of viral replication and the innate immune response of the host, provides a framework for the creation of prospective antiviral drugs. Zika virus methyltransferase helps in the catalysis of methylation for cap structure synthesis. The MTase domain increases NS5's affinity for binding the RNA template and incoming nucleotides, facilitating RdRp-mediated replication initiation and elongation. While assigning the cellular eIF4e factor (Eukaryotic translation initiation factor 4E) for translation initiation and shielding the viral RNAs from host innate immunity detection systems like RIG-I, MDA5, and interferon-induced restriction factors like IFIT (Interferon-induced proteins with tetratricopeptide repeats) molecules, the viral modifications play a crucial role in protecting the viral mRNAs against cellular 50-exonucleases.**(Bharadwaj et al., 2021)**

Interferons (IFN) are known to activate the following signaling pathways- type I, type II, type III ad type IV. IFN type II signaling has been implicated in ZIKV infection, but IFN type I and IFN type III signaling have been demonstrated to exert potent antiviral effects and be antagonized by ZIKV NS5). The NS5 protein is an intriguing target for antiviral treatment since it plays numerous roles in ensuring virus survival and reproduction. The hydrophobic cavity next to SAM binding site, which is conserved among flavivirus, or the positively charged RNA binding tunnel can both be used to increase the specificity of SAM analogs.**(Elshahawi et al., 2019)**

2.3 Bound Ligands:

SAH (S-adenosyl-L-homocysteine) and glycerol (GOL) are the ligands for ZIKV NS5 MTase, whereas sulfate and zinc ions act as a cofactor.

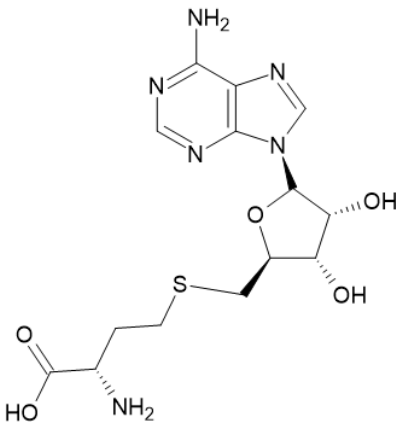


Figure 3- Representing the ligand, i.e., SAH (S-adenosyl-L-homocysteine).

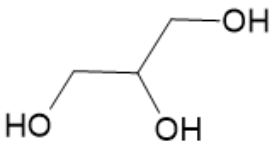
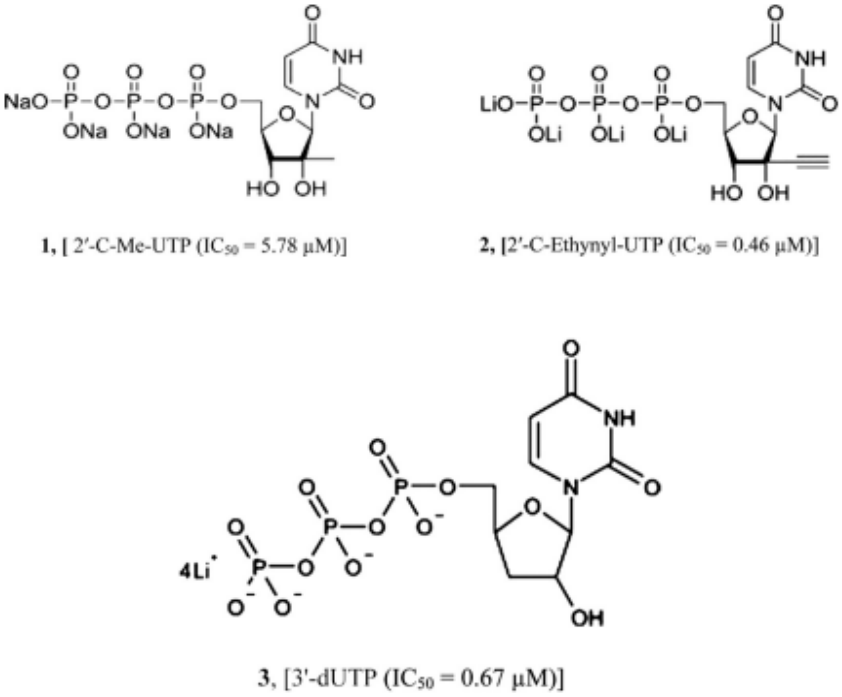


Figure 4- Representing the structure of ligand, i.e., glycerol.

2.4 Inhibitors-

(a) Known inhibitors: The following are the inhibitors for RdRp;



1, [2′-C-Me-UTP (IC_{50} = 5.78 µM)]

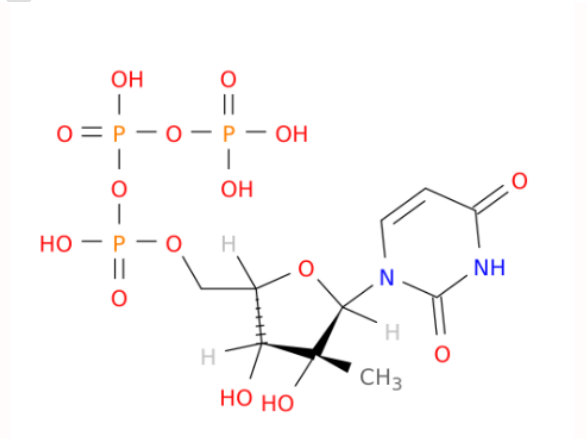
2, [2′-C-Ethynyl-UTP (IC_{50} = 0.46 µM)]

3, [3'-dUTP (IC_{50} = 0.67 µM)]

Figure 5: Known inhibitors of RdRp Proteins (Patil et al., 2019)

(b) Similar inhibitors:

1. **2'C-Me-UTP**



2. **2'C-Me-UTP**

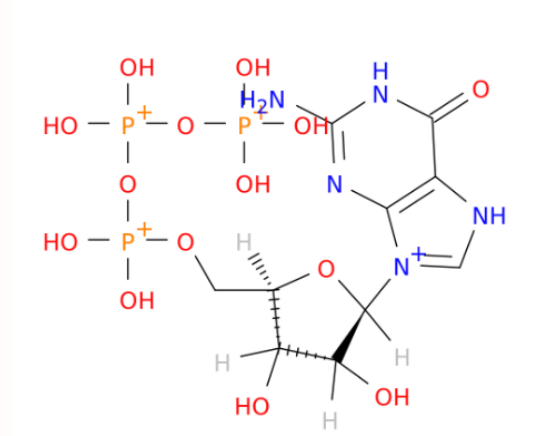


Figure 6: [(2R,3S,4R,5R)-5-(2-amino-6-oxo-1,7-dihydropurin-9-ium-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy-[dihydroxy(trihydroxyphosphaniumyloxy)phosphaniumyl]oxy-dihydroxyphosphanium

2.5 Docking and Virtual Screening

The structure of the receptor-ligand complex is predicted using molecular docking, wherein the receptor is often a protein or nucleic acid and the other protein or any small molecule acts as a ligand. **(Hernández-Santoyo et al., n.d.)** To anticipate the binding interactions for two or multiple molecules, computational docking is utilized. Two techniques are used in computational docking, initially, a force field is used to calculate the complex's free energy of binding, which is typically calculated based on the specifically bound conformation, and then, subsequently, a search approach to investigate the conformational area accessible to the ligand and the target. In order to quickly access the ligand-protein interaction during the docking simulation, a grid-based method is employed for energy evaluation, interaction energies are first estimated around the target structure. In order to confirm that the docking algorithm can accurately duplicate the observed binding mode, docking methods are frequently tested using 'redocking studies'.

A computer-based method referred to as "Virtual Screening" can be used to find the potential compounds that will bind to the target molecule with a known structure. This technique is becoming more and more popular for finding novel inhibitors and therapeutic compounds. The concept underlying virtual screening

is that a library of small compounds is docked into the binding pocket of a protein and then a number of solutions per molecule, among many of the top ranks, are restored. A decision is then made regarding the fraction of compounds to be moved forth for screening toward a hit identification. The fundamental idea is that virtual screening distinguishes active and inactive compounds, which eventually reduces the number of molecules and may provide a supplement to high-throughput screening (HTS).**(Cosconati et al., 2010)**

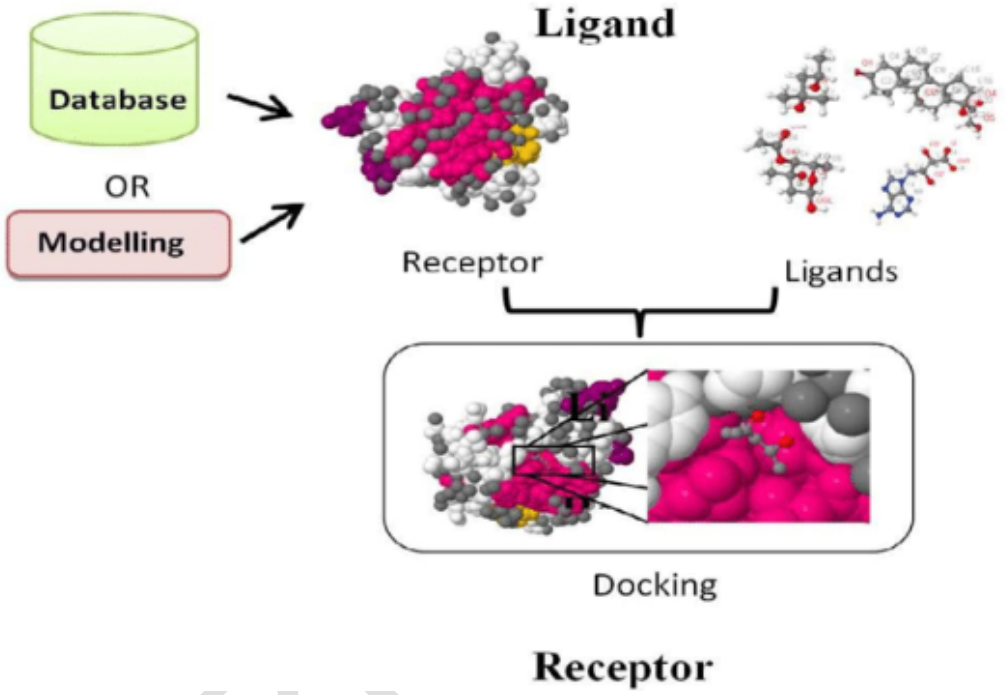


Figure 7- Representing the flowchart for molecular docking.(**Hernández-Santoyo et al., n.d.)**

3. Objectives-

3.1 To identify and enlist the proteins of the Zika Virus and select critical proteins for a potential target of the drug.

3.2 To analyze the structural characteristics of the target protein.

3.3 To identify the chemical compounds that can act as potential inhibitors by structure-based virtual screening performed against the ligand database

3.4. Identification of similar protein in Plant, animal, fungus and prokaryotic world.

3.5 Study of Evolutionary conservation or Phylogenetic Analysis of Protein.

3.6 Homology Based Modeling of similar proteins using the Zika virus protein as crystal structure template.

4. Materials and Methods

5. Results

(unpublished data)

REFERENCES:

Bharadwaj, S., Rao, A. K., Dwivedi, V. D., Mishra, S. K., & Yadava, U. (2021). Structure-based screening and validation of bioactive compounds as Zika virus methyltransferase (MTase) inhibitors through first-principle density functional theory, classical molecular simulation and QM/MM affinity estimation. *Journal of Biomolecular Structure and Dynamics*, *39*(7), 2338–2351. https://doi.org/10.1080/07391102.2020.1747545

Elshahawi, H., Syed Hassan, S., & Balasubramaniam, V. (2019). Importance of Zika Virus NS5 Protein for Viral Replication. *Pathogens*, *8*(4), 169. https://doi.org/10.3390/pathogens8040169

Zhang, C., Feng, T., Cheng, J., Li, Y., Yin, X., Zeng, W., Jin, X., Li, Y., Guo, F., & Jin, T. (2017). Structure of the NS5 methyltransferase from Zika virus and implications in inhibitor design. *Biochemical and Biophysical Research Communications*, *492*(4), 624–630. https://doi.org/10.1016/j.bbrc.2016.11.098

Shi, Y., & Gao, G. F. (2017). Structural Biology of the Zika Virus. *Trends in Biochemical Sciences*, *42*(6), 443–456. https://doi.org/10.1016/j.tibs.2017.02.009

Song, W., Zhang, H., Zhang, Y., Chen, Y., Lin, Y., Han, Y., & Jiang, J. (2021). Identification and Characterization of Zika Virus NS5 Methyltransferase Inhibitors. *Frontiers in Cellular and Infection Microbiology*, *11*, 665379. https://doi.org/10.3389/fcimb.2021.665379

Hernández-Santoyo, A., Tenorio-Barajas, A. Y., Altuzar, V., Vivanco-Cid, H., & Mendoza-Barrera, C. (n.d.). *Protein-Protein and Protein-Ligand Docking*. 20.

Cosconati, S., Forli, S., Perryman, A. L., Harris, R., Goodsell, D. S., & Olson, A. J. (2010). Virtual screening with AutoDock: Theory and practice. *Expert Opinion on Drug Discovery*, *5*(6), 597–607. https://doi.org/10.1517/17460441.2010.484460

Ramharack, P., & Soliman, M. (2018). Zika virus NS5 protein potential inhibitors: an enhanced in silico approach in drug discovery. *Journal of biomolecular structure & dynamics*, *36*(5), 1118–1133. https://doi.org/10.1080/07391102.2017.1313175

Figure 3: Molecular docking flow chart. (n.d.). ResearchGate. Retrieved July 10, 2022, from https://www.researchgate.net/figure/Molecular-docking-flow-chart_fig6_270822306

Patil, V. M., Masand, N., & Gupta, S. P. (2019). Zika Virus Polymerase. In *Viral Polymerases* (pp. 357–385). Elsevier. https://doi.org/10.1016/B978-0-12-815422-9.00013-9

Wang, B., Tan, X.-F., Thurmond, S., Zhang, Z.-M., Lin, A., Hai, R., & Song, J. (2017). The structure of Zika virus NS5 reveals a conserved domain conformation. *Nature Communications*, *8*(1), 14763. https://doi.org/10.1038/ncomms14763