



## Original Article

# 3D molecular structure analysis of NS2B/NS3 proteases derived from dengue virus and Zika virus

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## Abstract

Both dengue and Zika viruses have infected millions of people worldwide, urging the development of efficacious drugs and vaccines to fight the infection. Unfortunately, current research is yet to elucidate the structural proteomics comparison of the NS2B/NS3 from both viruses. Therefore, the main objective of this study was to comparatively study the structural proteins between dengue and Zika viruses by leveraging standard homology modelling tools. Our data provide 3D molecular structure overviews of NS2B/NS3 derived from the foregoing viruses. Sequence alignment indicated that the viruses share a 56% similarity rate of protein structures. However, in terms of function, both have NS2B that is pivotal for the activation of NS3 proteases.

**Keywords:** Dengue, Zika, NS2B/NS3, homology modelling, structural comparison

## Introduction

Dengue virus (DENV) and Zika virus (ZIKV) are single stranded RNA viruses that belong to the genus Flavivirus (Rawal et al., 2016; Back and Lundkvist, 2013). DENV and ZIKV are both mosquito-borne Flavivirus with similar transmission cycle, distribution throughout tropics and disease manifestations (Sharp et al, 2019). To reach the maturation of DENV particles would require a proper cleavage of the viral polyprotein. Hence, the cleavage will include the processing of eight of the thirteen substrate cleavage sites by dengue virus NS2B/NS3 protease and this is essential for maturation of the viral particle (Lin et al, 2017).

DENV NS2B/NS3 is a protease that belongs to the chymotrypsin, the proper function of NS3 that eventually participates in substrate recognition (Lin et al, 2017). While ZIKV contains a positive- sense single-stranded RNA genome that encodes a single 3419 amino acid forming a polypeptide, which is then cleaved to form three structural proteins and seven non- structural proteins (Bowen et al, 2019). In the case of ZIKV NS2B/NS3, its NS3 contains the catalytic triad, and along with the transmembrane NS2B will form the NS2B/NS3 protease that is critical to the replication and survival of ZIKV (Bowen et al, 2019). Both viruses have NS2B/NS3 protease that plays a critical function in the development, replication and survival of the viral particle. Thus, inhibition of NS2B/NS3 protease is a promising target for drug development.

The main goal in this study is to gain the insight on the differences of NS2B/NS3 proteases from between DENV and ZIKV by identifying the similarity and differences between the structure. Herein, the 3D structure analysis on NS2B/NS3 proteases from both viruses was performed by a visualization through a molecular graphic software.

## Method

Data used in this study were supplied from NCBI's Molecular Modeling Database (MMDB) based on the Protein Data Bank (PDB) and Vector Alignment Search Tool (VAST) (Madej et al, 2014). In this research, VAST will be used alongside with the

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VAST+. VAST+ is the enhanced version of VAST which is able to generate a report of the structure neighbours with the aim to find the largest set of pairs of matching macromolecules between two biological assemblies, characterize the match and compute instructions to visualize the structural similarity (Madej et al, 2014). In this research, comparative analysis was performed between both proteases in terms of molecular structure, specifically at the level of primary and secondary structure of the enzymes.

The data were downloaded after a search on the Protein Data Bank (PDB) using the terms of Dengue Virus NS2B/NS3 Protease (PDB ID: 2FOM; <https://www.rcsb.org/structure/2FOM>) and Zika virus NS2B/NS3 Protease (PDB ID: 5GXJ; <http://www.rcsb.org/structure/5GXJ>). 2FOM was subjected to search for similar structures by using VAST. The subsequent search generated all matching molecules superposed. We eliminated the rest of the results and only consider the 5GXJ for direct comparison. Visualization of the 3D structure superposition and result of sequence alignment were observed on iCn3D (a web-based 3D structure viewer provided by NCBI).

## Results and Discussion

Correct processing by the NS3 protease during DENV or ZKV replication is essential. Overall, NS3 protease domains of DENV follow a chymotrypsin-like fold with two  $\beta$ -barrels with each formed by six  $\beta$ -strand alongside catalytic triad located at the cleft between two beta-barrels (Figure 1A). Herein, the overall structure of DENV NS3 is indicated by blue colour strand while the NS2B – purple colour (Figure 1A). According to the previous study on the structure of DENV NS2B/NS3 proteases, the electron density beyond NS2B residue 76 is discontinuous, indicating the C-terminal part of the cofactor might adopt multiple conformations in solution (Erbel et al, 2006). Through the observation in this present study, the structure of DENV and ZIKV NS2B/NS3 proteases are not quite similar. The structure of 5GXJ itself has been published either on the PDB or NCBI. On contrary, the literature regarding 2FOM structure is currently yet published. Therefore, herein, the 5GXJ is described briefly according to the information generated by NCBI's MMDB.

Comparison of both structure shows the difference in terms of polyprotein, especially on their lengths. For example, the polyprotein chain A of 2FOM (purple colour, refers to the NS2B) has the length of 62 amino acids (Figure 1A), while the same polyprotein chain A of 5GXJ has the length of 224 amino acids (Figure 1B). Other than that, even though 2FOM and 5GXJ have the same region of NS2B and peptidase, the difference could be observed on their position. In 2FOM, NS2B is located in the polyprotein A chain with the subsequent peptidase located inside the polyprotein B chain. While in the case of 5GXJ, as both chains have the same length (224 amino acids), the NS2B as well as the peptidase are located within the same region of aforementioned polyprotein chains.

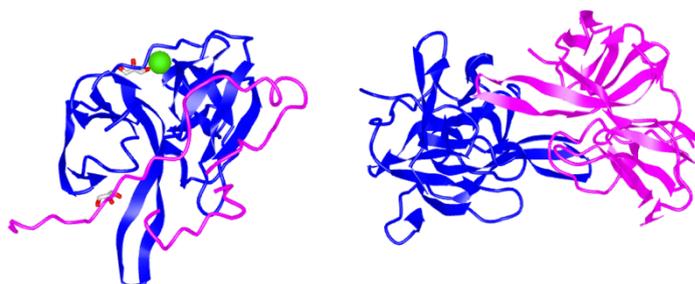


Figure 1. (A) 3D dimeric structure of DENV NS2B/NS3 Proteases (2FOM) and (B) 3D dimeric structure of ZIKV NS2B/NS3 Proteases (5GXJ).

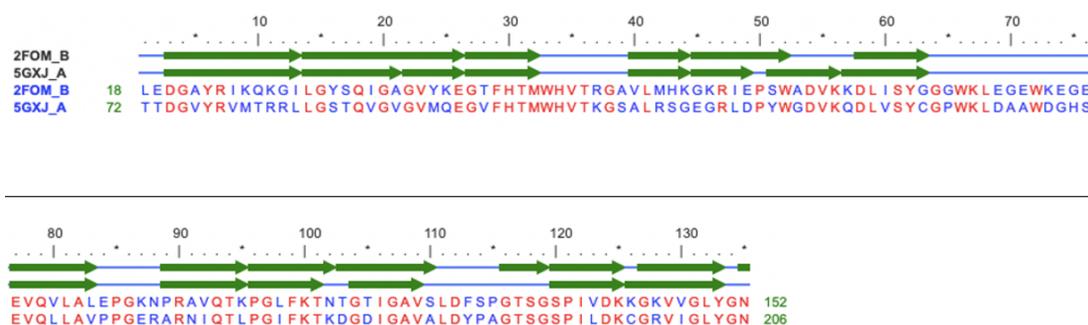


Figure 2. Alignment result of DENV NS2B/NS3 protease (2FOM) and ZIKV NS2B/NS3 protease (5GXJ).

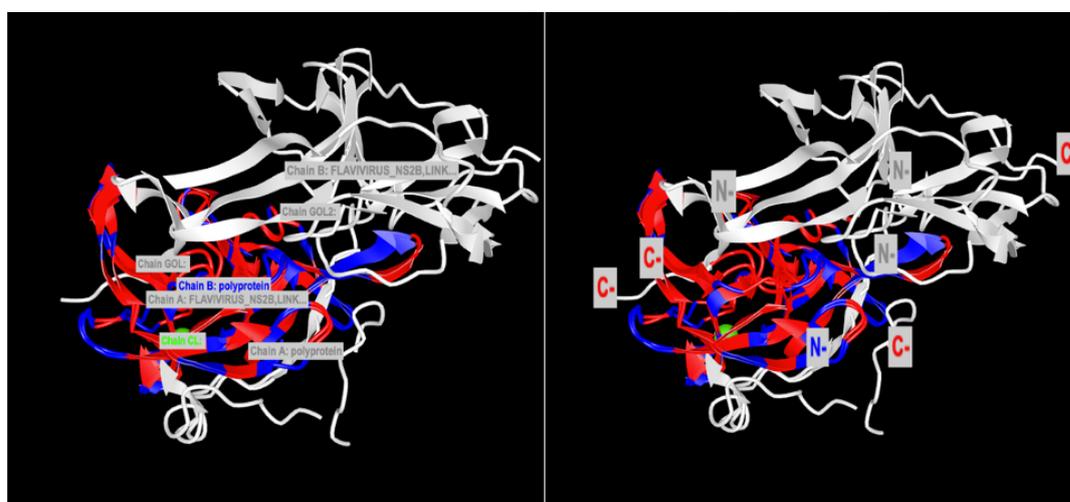


Figure 3. (A) 3D structure of the ensuing alignment 2FOM and 5GXJ with the addition of label chain. (B) Location of N- and C-terminal from the alignment of 2FOM and 5GXJ.

Sequence alignment was subjected to both structure in order to gain insight on similarity between the two structures which then could be visualize the 3D structure superposition (Figure 2). The subsequent alignment only takes the polyprotein chain B of 2FOM and polyprotein chain A of 5GXJ as the main template for the alignment process. Alignment result showed there are 60 mismatches of amino acids out of 135 that are subjected to the alignment process. Thus, making up the similarity only just 56%. 3D structure analysis depicting the result of the alignment have been presented (Figure 3A,B). As we can see, the combination of blue and red colour ribbon indicates the successful alignment of polyprotein B chain of 2FOM with polyprotein A chain of 5GXJ. This result shows the polyprotein B chain of 5GXJ are likely unaligned due to the difference in the length with its counterpart (Figure 3A). Hence, this possibly would be the answer why the similarity of both structures only reaches 56%. The contribution of NS2B itself in the formation of an active protease among the Flaviviridae family differs substantially especially those observed with other cofactor- activated viral proteases (Erbel et al, 2006). However, in regards to the NS2B itself, it completes the substrate-binding site with its C-terminal region and contribute to a stability in both N- and C-terminal by creating additional  $\beta$ -strands (Figure 3B) (Erbel et al, 2006).

This study provides a 3D molecular structure overview of NS2B/NS3 derived from both DENV (2FOM) and ZIKV (5GXJ). Overall, both structures are not similar as from the sequence alignment, the rate of similarity is only just 56%. However, in

terms of function, both have NS2B that is pivotal for the activation of NS3 proteases. However, lot of limitations are identified in this study. First, we only conduct a one-on-one comparison between NS2B/NS3 of DENV and ZIKV. This one-on-one comparison may cause bias in the analysis as different structures that came from the same viruses are available in the NCBI's MMDB. Second, indeed 2FOM already previously studied since 2006 by Erbel et al, however, the 5GXJ from ZIKV that was used in this study have no publications yet and therefore, the ensuing analysis might be questionable. Third, we did not perform molecular docking simulation to compare the difference on how both NS2B/NS3 protease bind with a specific substrate.

## Conclusion

NS2B/NS3 proteases are the integral part in Flavivirus. Their replication would require the correct processing of their subsequent polyprotein by NS3 protease. In this case, NS2B is important as the activator of NS3. These studies only focus on the 3D molecular structure analysis of NS2B/NS3 derived from both viruses. For future research, we recommend to perform the study employing DENV and ZKV variants. Furthermore, molecular docking simulation could be performed to observe the the ability of NS2B/NS3 proteases to bind with a specific substrate.

## Authors' contributions

Conceptualization: SB and AAP; Data curation: SB and AAP; Formal analysis: SB and AAP; Investigation: SB and AAP; Methodology: AAP; Resources: SB; Supervision: SB; Validation: AAP; Writing-original draft preparation: SB and AAP; Writing-review and editing: SB and AAP.

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## Conflict of interest

There is no conflict of interest was reported by the authors.

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