Malaysian Journal of Medical Research, 10(1), 105-119
https://doi.org/10.31674/mjmr.2026.v010i01.012

MJMR
Malaysian Journal of Medical Research

Malaysian Journal of Medical Research
Online ISSN: 2550-1607
www.mjmr.com.my

Original Article

A Bioinformatic Approach to Designing a Mosaic Dengue Vaccine with Optimized Immunoreactivity and Safety

Muhammad Asaduzzaman[1]*, Md. Ahashan Habib[1], Rakib Hossain[2], Mst Umme Hanya[3], Urmila Rahman[4]

[1]Directorate General of Health Services, Mohakhali, Dhaka 1212, Bangladesh
[2]Khulna University, Sher-e-Bangla Road, Gollamari, Khulna-9208, Bangladesh
[3]Datascape Research and Consultancy Limited, Main Road, Block A, Mirpur-11, Dhaka-1216, Bangladesh
[4]Dr. Sirajul Islam Medical College & Hospital Ltd, New Circular Road, Moghbazar, Dhaka-1217, Bangladesh

Corresponding Author's Email: net.jmc10@gmail.com

Abstract

Background: Dengue virus infects approximately 390 million people annually and exists as four distinct serotypes. The only licensed dengue vaccine has demonstrated variable efficacy, showing reduced protection in seronegative individuals and against dengue virus serotypes 1 and 2. These limitations highlight the need for improved vaccine strategies that better represent dengue antigenic diversity. Objective: This study aimed to design and evaluate a mosaic vaccine strategy based on the dengue virus envelope (E) protein that could represent conserved antigenic regions across all four serotypes and potentially improve vaccine efficacy. **Methods:** A novel mosaic E protein composed of nine conserved fragments from dengue virus E proteins was designed using bioinformatics approaches. Sequence similarity analyses were performed to compare the mosaic protein with wild-type E proteins from each serotype and to assess homology with human proteins or other pathogens. Predicted secondary (2D) and tertiary (3D) protein structures were analyzed to determine whether the mosaic protein would mimic the natural E protein structure. Immunoinformatic analyses were conducted to evaluate antigenicity and immunogenicity. **Results:** The mosaic E protein demonstrated high similarity (>95%) to the wild-type E proteins of all four dengue serotypes. Secondary structure analysis showed a predominance of β-sheet structures, consistent with the flexibility observed in natural E proteins. High-scoring predicted 3D models exhibited acceptable global (>0.4) and local model quality scores, supporting structural reliability. Immunoinformatic analyses indicated that the mosaic proteins had similar or enhanced antigenicity and immunogenicity compared to the native E proteins. **Conclusion:** The mosaic E protein vaccine strategy effectively represents conserved antigenic diversity across dengue virus serotypes within a single recombinant protein. These findings suggest that this approach holds promise as a next-generation dengue vaccine candidate and warrants further experimental validation in laboratory settings.

Keywords: *Bioinformatic analysis; Dengue; Envelope; Immunoinformatic; Mosaic; Vaccine*

Introduction

Dengue is a viral illness transmitted by Aedes aegypti mosquito is the most prevalent among the diseases regarded as neglected tropical disease by World Health Organization (WHO). Given the substantial global disease burden and the limitations of existing vaccines, immunoinformatic-driven mosaic vaccine design offers a promising strategy to achieve broad, serotype-independent protection

Received: 23thSeptember 2025; Revised version received on: 29th December 2025; Accepted: 30th January 2025

(Dixit, 2021; Fadaka *et al.*, 2021). Such an epitope-based tetravalent construct leverages peptide specificity to avoid pathogen interference while enhancing immunogenic breadth, as demonstrated by recent immunoinformatic pipelines that integrate B-cell and T-cell epitope prediction, molecular docking, and in-silico immune simulation. Indeed, recent dengue studies have pinpointed highly conserved B-cell epitopes across all four serotypes that can be incorporated into a mosaic construct to enhance breadth and safety (Chan *et al.*, 2020), while immunoinformatic frameworks that fuse epitopes with appropriate linkers and perform codon optimization have proven effective for rapid vaccine prototyping (Aziz *et al.*, 2022).The global incidence of dengue is spiking at an alarming rate with almost about 400% increase over just 13 years (2000-2013) (Fitzmaurice *et al.*, 2017). Although, once it was restricted to only tropical and subtropical countries but nowadays it is spreading worldwide as evidenced by a recent data published by Pan American Health Organization showing that countries in American region has faced a record breaking 2.7 million new cases and 1206 deaths only in 2019 between the months from January to October and recent numbers of explosive outbreak are occurring worldwide such as very recent outbreaks of 2016 and 2019 (WHO, 2019a; WHO, 2019b). Consequently, leveraging multi-epitope immunoinformatic pipelines—successfully applied to Hantaan virus, SARS-CoV-2, and other pathogens—offers a rapid, cost-effective route to engineer a dengue mosaic vaccine with broad serotype coverage and improved safety profile (Ghafoor *et al.*, 2021; Invenção *et al.*, 2025; Khalid *et al.*, 2022; Rasheed *et al.*, 2023).

About half of the world's population is now in risk of being affected by dengue with around 390 million of annual incidence among which almost 96 million people develop clinical manifestations (Bhatt *et al.*, 2013). Dengue shows wide spectrum of presentations ranging from subclinical asymptomatic infections to hypovolemic shock and organ failure. Based on clinical manifestations dengue is classified as dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Ajlan *et al.*, 2019). DHF presents with haemorrhage and thrombocytopaenia of different extent whereas DSS manifests as vascular leakage leading to hypovolemic shock and multi-organ failure. Both DHF and DSS are regarded as severe dengue in newer classification proposed by World Health Organisation (WHO) in 2009 (Ajlan *et al.*, 2019). Recovery from an infection provide lifelong homotypic immunity to the causative serotype. However, cross immunity to other serotypes is usually partial and transient. Secondary infection by a different serotype may instead result in severe dengue which is mediated by a mechanism known as antibody dependent enhancement (ADE)which occurs due to lower level of neutralising antibodies in the circulation and/or presence of cross reactive non-neutralising antibodies which in turn cause immature opsonisation and enhances FCRγ mediated internalisation of virus particles (Whitehorn & Simmons, 2011). Consequently, any vaccine candidate must elicit robust, serotype-balanced neutralizing antibodies to prevent antibody-dependent enhancement and ensure safe, cross-protective immunity (Perez *et al.*, 2025). Consequently, pre-clinical assessment of the mosaic construct in appropriate animal models will be crucial to verify its immunogenicity, safety profile, and ability to induce balanced neutralizing antibodies against all four serotypes (Hou *et al.*, 2019; Kaushik *et al.*, 2022). Some studies also postulate that cells infected via ADE are immunomodulated in such a way that their intracellular environment favour viral replication (Ubol & Halstead, 2010). Thus, incorporating ADE assessment into preclinical testing is essential, and recent mRNA-LNP vaccine data demonstrate potent neutralizing immunity with minimal ADE risk (He *et al.*, 2022). These antibodies not only promote viral replication but also activate mast cell by FCRγ dependent pathway and thereby inducing vascular pathology (Wan *et al.*, 2018). There is no definite treatment available for dengue till now and only one licensed vaccine, Dengvaxia; developed by Sanofi Pasteur is in the market. But frustratingly Dengvaxia has been shown to increase the risk of developing severe dengue in patients who were seronegative at the time of vaccination and also in children under the age of 5 years (Halstead, 2018). Two other vaccines namely Takeda's TAK-003 made up on DENV-2 backbone with DENV-DENV chimera and Instituto Bhutantan's Bhutanatan-DV; a live attenuated vaccine made by mutagenesis with one DENV-DENV chimera are in phase-3 clinical trial (WHO, 2017). Among them Takeda has recently published its result of part-1 of a multinational phase-3 clinical trial involving 20,071 participants aging between 4 to 16 years where it showed overall vaccine efficacy of 80.9%. However, vaccine efficacy was lower in basal seronegative participants which is a major concern (Biswal *et al.*, 2019) and long-term safety of the vaccine is still now questionable. (Dixit, 2021; Fadaka *et al.*, 2021)

Like other RNA virus dengue virus are also highly mutagenic and some studies also postulate that reinfection with same serotype is possible owing to this hyper-mutagenicity, making vaccine design against the virus more challenging. To overcome this challenge Hou *et al*. have utilized the 'Mosaic vaccine' strategy to develop a DNA vaccine using the precursor membrane (Prm) and envelope (ENV) gene sequences from each serotype that produced some promising results by provoking cell mediated immunity in addition with humoral immunity (Hou *et al.*, 2019). Future investigations should assess the mosaic construct's capacity to elicit durable, serotype-balanced T-cell responses in relevant animal models. To further investigate the strategy, it will perform the bioinformatic analysis of mosaic design of envelope protein to evaluate its potential as a vaccine candidate. Subsequent in silico epitope conservancy and population-coverage analyses will further substantiate its suitability for broad-scale immunization (Ambuel *et al.*, 2014).

Methodology

Data Retrieval

All of the primary sequences were collected from Virus Pathogen Database and Analysis Resource (ViPR) database (Virus Pathogen Database and Analysis Resource, 2020). First, Flaviviridae taxon was selected followed by selection of flavivirus genus, dengue virus species and respective serotype. Only the complete sequences were retrieved. Sequence of a control strain for each serotype was retrieved which were from Bangladesh for dengue virus- 1,2 and 3. But as no sequence was found for serotype 4 in Bangladesh so it was collected from an Indian isolate as India and Bangladesh are neighbouring country and share much of the topographic similarity.

Generation of Mosaic Sequences

Mosaic sequences for each serotype were obtained using all the complete sequences of envelop protein retrieved from ViPR databases for that particular serotype. These sequences were then submitted to the mosaic vaccine designer tool of the https://hfv.lanl.gov/scratch/MOSAIC server.

Alignments

Sequence of the mosaic envelop protein was compared with the envelop protein sequence of the control strain for each serotype utilising the BLASTP sever. To run the alignment multiple sequence alignment (MSA) programme was used to input the mosaics as query and control strains as subject sequence and BLASTP was set as default algorithm and the method used was Compositional matrix adjust (Altschul *et al.*, 1997). The mosaic sequences were also aligned against each other to see the similarity between them using both constraint-based alignment tool for multiple protein sequences (COBALT) program of BLAST and CLUSTALW server (16, 17).

Homology Detection

To search for homology HHpred server; an integrated bioinformatic server with pristine sensitivity for detecting remote homologues by utilising pairwise comparison of profile hidden Markov models (HMMs), was used and all four mosaic sequences were submitted individually as input to the server. HHpred produces superior results to other search database by searching through alignment databases, like Pfam or SMART instead of sequence databases such as UniProt or the NR. To run the program structural or domain database was selected as PDB_mmCIF70 which is updated up to 23rd July of 2020. All the parameters were set as default (Zimmermann *et al.*, 2018).

Protein Structure Prediction

The 3D structure of the mosaic proteins was acquired by using the latest version of the IntFOLD server (5.0); an integrated protein structure predicting and analysing tool (19, 20). The 2D structure properties was analysed by 2struc server; an online based opensource server. The disorders of the predicted 3D model were sorted out using DISOclust program (McGuffin, 2008).

Immunoinformatic Analysis

To evaluate the immunogenicity of the mosaic proteins, presence of epitopes for cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL) and B cell was examined. For prediction of CTL specific epitope NETCTL server was used while for HTL MHC2MIL server and for B cell was used. The antigenicity and toxicity of the proteins was also eventually predicted using vexijen2.0 server and toxinpred sever respectively. Same procedures were followed to analyse the immunoinformatic properties envelop protein of control strains.

Results

Collected Sequences

There were 2155 complete protein sequence for envelop protein of dengue-1 serotype in the ViPR database as of 31st July 2020. For serotype 2, 3 and 4 the number was 1669, 987 and 252 respectively. For serotype 1 two complete envelop protein sequence were found as Bangladeshi isolate in the database and between them a sequence was collected as control strain; bearing the strain name B17-1387 and GenBank protein accession number 'VIPR_ALG4_BBH51315_1_915_2399.1'. In case of serotype 2 the selected strain, among the seven found in the database, was B17-1357 with GenBank protein accession number 'VIPR_ALG4_BBH51317_1_909_2393.1', while B17-1471 with GenBank protein accession number VIPR_ALG4_BBH51324_1_915_2393.1 was selected among 10 strains found in the database. No serotype 4 was isolated in Bangladesh so an Indian isolate DENV-4/IND/0952326/2009 with GenBank protein accession number VIPR_ALG4_428625154_935_2419.1 was selected as control for this serotype.

Mosaic Sequences

Following submission of all the sequences for each serotype (i.e.: 2155 for serotype 1, 1669 for 2, 987 for 3 and 252 for 4) separately in the https://hfv.lanl.gov/scratch/MOSAIC server, it revealed a mosaic sequence for the respective serotype. The mosaic sequences are illustrated in the Figure-1.

a

```
MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEV
TNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRG
WGNGCGLFGKGSLITCAKFKCVTKLEGKIVQYENLKYSVIVTVHTGDQHQ
VGNESTEHGTTATITPQAPTTEIQLTDYGALTLDCSPRTGLDFNEMVLLT
MKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTFKTAHAKKQEV
VVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLTLKGMSYV
MCTGSFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQDEKGVTQNGR
LITANPIVTDKEKPVNIEAEPPFGESYIVIGAGEKALKLSWFKKGSSIGK
MFEATARGARRMAILGDTAWDFGSIGGVFTSVGKLVHQIFGTAYGVLFSG
VSWTMKIGIGVLLTWLGLNSRSTSLSMTCIAVGLVTLYLGVMVQA
```

b

```
MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEA
KQPATLRKYCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRG
WGNGCGLFGKGGIVTCAMFTCKKNMEGKIVQPENLEYTIVITPHSGEEHA
VGNDTGKHGKEIKVTPQSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ
MENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDV
VVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYS
MCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGR
LITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQ
MFETTMRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSG
VSWTMKILIGVIITWIGMNSRSTSLSVSLVLVGVVTLYLGVMVQA
```

c

```
MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEA
TQLATLRKLCIEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRG
WGNGCGLFGKGSLVTCAKFQCLEPIEGKVVQYENLKYTVIITVHTGDQHQ
VGNETQGVTAEITPQASTTEAILPEYGTLGLECSPRTGLDFNEMILLTMK
NKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKNAHAKKQEVVV
LGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLELKGMSYAMC
TNTFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLI
TANPVVTKKEEPVNIEAEPPFGESNIVIGIGDNALKINWYKKGSSIGKMF
EATARGARRMAILGDTAWDFGSVGGVLNSLGKMVHQIFGSAYTALFSGVS
WVMKIGIGVLLTWIGLNSKNTSMSFSCIAIGIITLYLGAVVQA
```

d

```
MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTA
KEVALLRTYCIEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRG
WGNGCGLFGKGGVVTCAKFSCSGKITGNLVQIENLEYTVVVTVHNGDTHA
VGNDTSNHGVTATITPRSPSVEVKLPDYGELTLDCEPRSGIDFNEMILMK
MKKKTWLVHKQWFLDLPLPWTAGADTSEVHWNYKERMVTFKVPHAKRQDV
TVLGSQEGAMHSALAGATEVDSGDGNHMFAGHLKCKVRMEKLRIKGMSYT
MCSGKFSIDKEMAETQHGTTVVKVKYEGAGAPCKVPIEIRDVNKEKVVGR
VISATPLAENTNSVTNIELEPPFGDSYIVIGVGNSALTLHWFRKGSSIGK
MFESTYRGAKRMAILGETAWDFGSVGGLFTSLGKAVHQVFGSVYTTMFGG
VSWMIRILIGFLVLWIGTNSRNTSMAMTCIAVGGITLFLGFTVQA
```

Figure 1: Mosaic Sequence of Dengue Virus. a- Serotype 1, b- Serotype 2, c- Serotype-3 and d- Serotype 4

Alignment

The acquired mosaic sequences were aligned with the envelop protein sequence of respective control strain by BLASTP (Altschul *et al.*, 1997). The resultant alignment between mosaic and control strain of serotype 1 showed 97% identity similarity while for serotype 2,3 and 4 identities were 99%, 97% and 96% respectively. None of the alignment produced any gap (figure-2) (Altschul *et al.*, 1997). The mosaic sequences were also aligned with each other to check the similarity among them using COBALT software of BLAST server, which revealed much variation among them (figure-3) (Papadopoulos & Agarwala, 2007). The similarity scores among mosaic sequences as measured by CLUSTALW are as following (Thompson *et al*., 1994).

Mosaic 1: Mosaic 2 (Aligned). Score: 68.6869

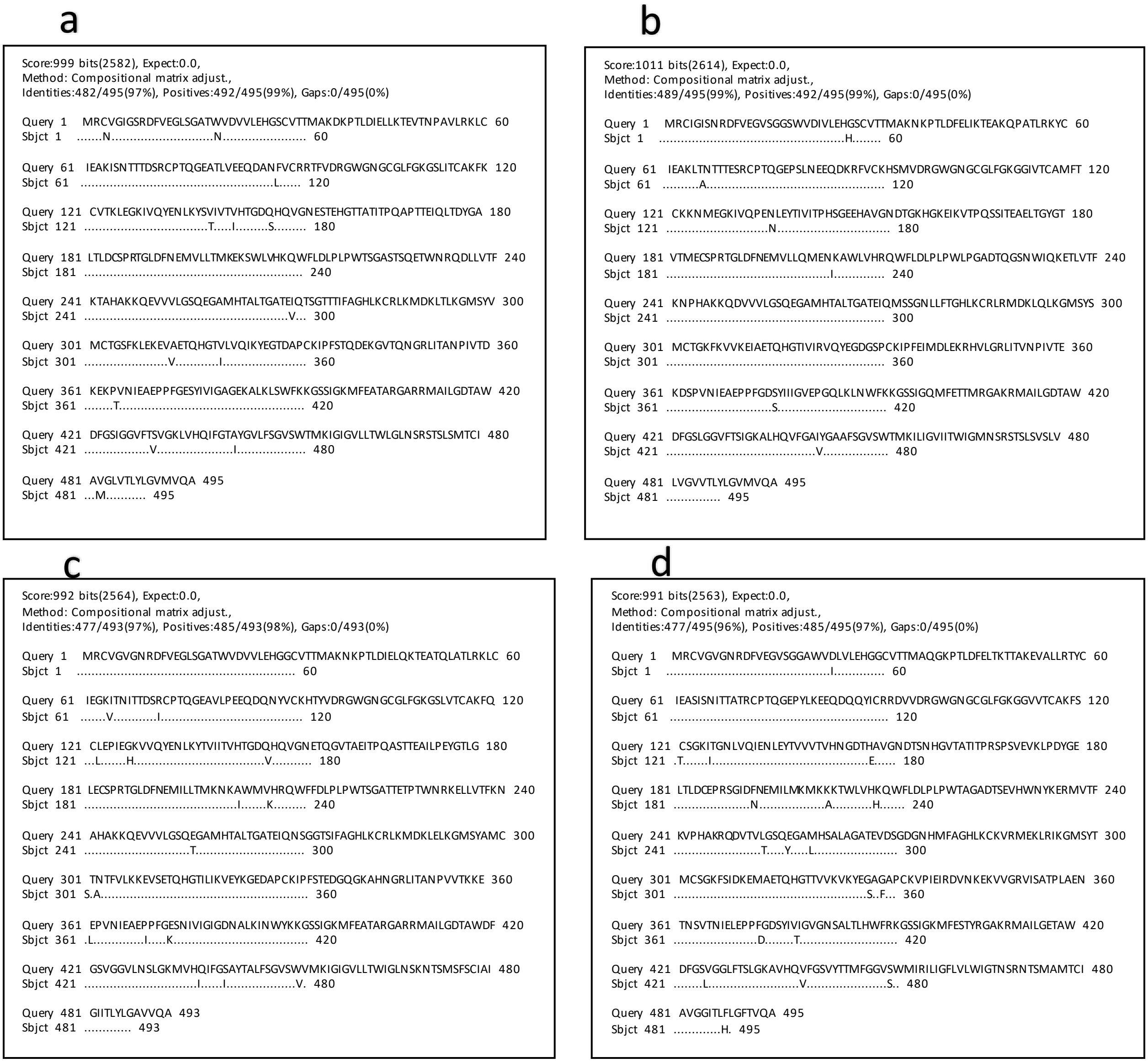
Mosaic 1: Mosaic 3 (Aligned). Score: 77.8905

Mosaic 1: Mosaic 4 (Aligned). Score: 63.8384

Mosaic 2: Mosaic 3 (Aligned). Score: 68.1542

Mosaic 2: Mosaic 4 (Aligned). Score: 63.6364

Mosaic 3: Mosaic 4 (Aligned). Score: 62.8803



a

```
Score:999 bits(2582), Expect:0.0,
Method: Compositional matrix adjust.,
Identities:482/495(97%), Positives:492/495(99%), Gaps:0/495(0%)

Query  1    MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEVTNPAVLRKLC  60
Sbjct  1    .......N...........................N......................  60

Query  61   IEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK  120
Sbjct  61   ..................................................L......  120

Query  121  CVTKLEGKIVQYENLKYSVIVTVHTGDQHQVGNESTEHGTTATITPQAPTTEIQLTDYGA  180
Sbjct  121  .................................T.....I.........S.........  180

Query  181  LTLDCSPRTGLDFNEMVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTF  240
Sbjct  181  ...........................................................  240

Query  241  KTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLTLKGMSYV  300
Sbjct  241  .......................................................V...  300

Query  301  MCTGSFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQDEKGVTQNGRLITANPIVTD  360
Sbjct  301  .......................V...........I.......................  360

Query  361  KEKPVNIEAEPPFGESYIVIGAGEKALKLSWFKKGSSIGKMFEATARGARRMAILGDTAW  420
Sbjct  361  ........T..................................................  420

Query  421  DFGSIGGVFTSVGKLVHQIFGTAYGVLFSGVSWTMKIGIGVLLTWLGLNSRSTSLSMTCI  480
Sbjct  421  ..................V.....................I..................  480

Query  481  AVGLVTLYLGVMVQA  495
Sbjct  481  ...M...........  495
```

b

```
Score:1011 bits(2614), Expect:0.0,
Method: Compositional matrix adjust.,
Identities:489/495(99%), Positives:492/495(99%), Gaps:0/495(0%)

Query  1    MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYC  60
Sbjct  1    ...................................................H........  60

Query  61   IEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFT  120
Sbjct  61   ..........A.................................................  120

Query  121  CKKNMEGKIVQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKVTPQSSITEAELTGYGT  180
Sbjct  121  ............................N..............................  180

Query  181  VTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTF  240
Sbjct  181  ............................................I..............  240

Query  241  KNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYS  300
Sbjct  241  ...........................................................  300

Query  301  MCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTE  360
Sbjct  301  ...........................................................  360

Query  361  KDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAW  420
Sbjct  361  ............................S..............................  420

Query  421  DFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLV  480
Sbjct  421  .........................................V..................  480

Query  481  LVGVVTLYLGVMVQA  495
Sbjct  481  ...............  495
```

c

```
Score:992 bits(2564), Expect:0.0,
Method: Compositional matrix adjust.,
Identities:477/493(97%), Positives:485/493(98%), Gaps:0/493(0%)

Query  1    MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEATQLATLRKLC  60
Sbjct  1    ...........................................................  60

Query  61   IEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQ  120
Sbjct  61   .......V............I.......................................  120

Query  121  CLEPIEGKVVQYENLKYTVIITVHTGDQHQVGNETQGVTAEITPQASTTEAILPEYGTLG  180
Sbjct  121  ...L.......H....................................V...........  180

Query  181  LECSPRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTFKN  240
Sbjct  181  ..........................................I.......K.........  240

Query  241  AHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLELKGMSYAMC  300
Sbjct  241  .............................T.............................  300

Query  301  TNTFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTKKE  360
Sbjct  301  S.A.........................................................  360

Query  361  EPVNIEAEPPFGESNIVIGIGDNALKINWYKKGSSIGKMFEATARGARRMAILGDTAWDF  420
Sbjct  361  .L..............I.....K.....................................  420

Query  421  GSVGGVLNSLGKMVHQIFGSAYTALFSGVSWVMKIGIGVLLTWIGLNSKNTSMSFSCIAI  480
Sbjct  421  ................................I......I...................V.  480

Query  481  GIITLYLGAVVQA  493
Sbjct  481  .............  493
```

d

```
Score:991 bits(2563), Expect:0.0,
Method: Compositional matrix adjust.,
Identities:477/495(96%), Positives:485/495(97%), Gaps:0/495(0%)

Query  1    MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTAKEVALLRTYC  60
Sbjct  1    ............................................I..............  60

Query  61   IEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGNGCGLFGKGGVVTCAKFS  120
Sbjct  61   ............................................................  120

Query  121  CSGKITGNLVQIENLEYTVVVTVHNGDTHAVGNDTSNHGVTATITPRSPSVEVKLPDYGE  180
Sbjct  121  .T.......I............................................E......  180

Query  181  LTLDCEPRSGIDFNEMILMKMKKKTWLVHKQWFLDLPLPWTAGADTSEVHWNYKERMVTF  240
Sbjct  181  ......................N..................A...........H.......  240

Query  241  KVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDGNHMFAGHLKCKVRMEKLRIKGMSYT  300
Sbjct  241  ........................T.....Y.....L......................  300

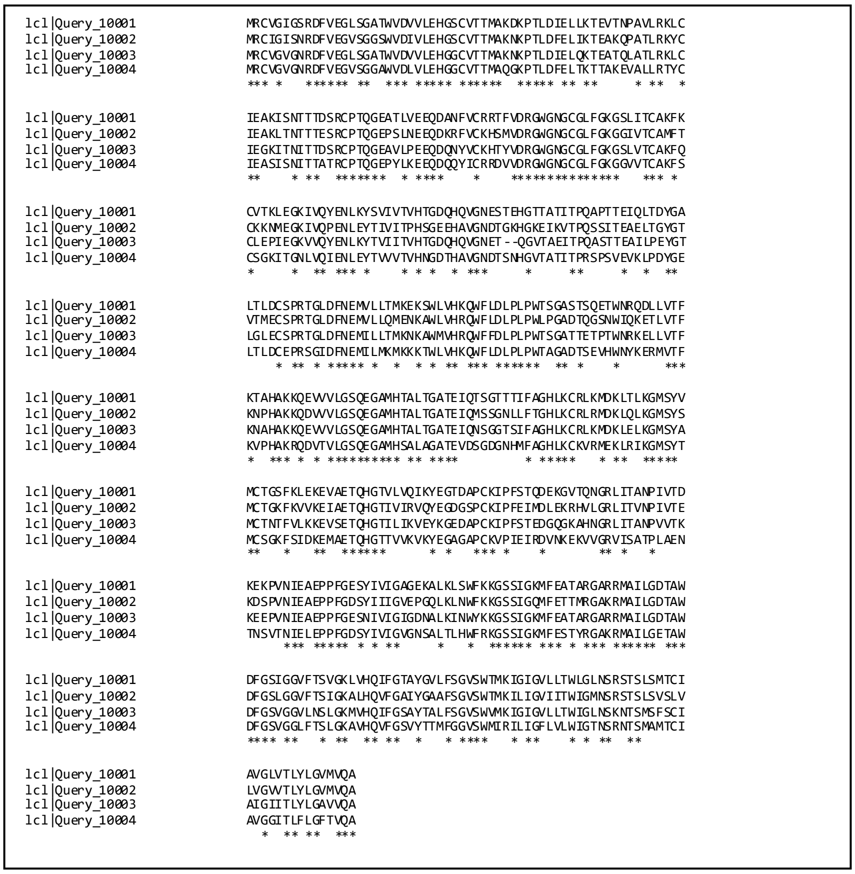
Query  301  MCSGKFSIDKEMAETQHGTTVVKVKYEGAGAPCKVPIEIRDVNKEKVVGRVISATPLAEN  360
Sbjct  301  ...................................................S..F...  360

Query  361  TNSVTNIELEPPFGDSYIVIGVGNSALTLHWFRKGSSIGKMFESTYRGAKRMAILGETAW  420
Sbjct  361  .........................D........T..........................  420

Query  421  DFGSVGGLFTSLGKAVHQVFGSVYTTMFGGVSWMIRILIGFLVLWIGTNSRNTSMAMTCI  480
Sbjct  421  ........L.......................V.........................S..  480

Query  481  AVGGITLFLGFTVQA  495
Sbjct  481  .............H.  495
```

Figure 2: Alignment between Mosaic Sequence and Corresponding Envelop Sequence of Control Strain. a – Alignment for Serotype 1, b – Alignment for Serotype 2, c – Alignment for Serotype 3, d – Alignment for Serotype 4. To See The Results Pairwise Alignment with Dots for Identity Was Selected (Altschul et al., 1997)



```
lcl|Query_10001               MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEVTNPAVLRKLC
lcl|Query_10002               MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYC
lcl|Query_10003               MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEATQLATLRKLC
lcl|Query_10004               MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTAKEVALLRTYC
                              *** *   ****** **  *** ***** ******  ***** ** **     * **  *

lcl|Query_10001               IEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK
lcl|Query_10002               IEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFT
lcl|Query_10003               IEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQ
lcl|Query_10004               IEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGNGCGLFGKGGVVTCAKFS
                              **    * **  *******  * ****    *    ***************   *** *

lcl|Query_10001               CVTKLEGKIVQYENLKYSVIVTVHTGDQHQVGNESTEHGTTATITPQAPTTEIQLTDYGA
lcl|Query_10002               CKKNMEGKIVQPENLEYTIVITPHSGEEHAVGNDTGKHGKEIKVTPQSSITEAELTGYGT
lcl|Query_10003               CLEPIEGKVVQYENLKYTVIITVHTGDQHQVGNET--QGVTAEITPQASTTEAILPEYGT
lcl|Query_10004               CSGKITGNLVQIENLEYTWVVTVHNGDTHAVGNDTSNHGVTATITPRSPSVEVKLPDYGE
                              *     *  ** *** *    * * *  * ***     *     **     *  *  **

lcl|Query_10001               LTLDCSPRTGLDFNEMVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWNRQDLLVTF
lcl|Query_10002               VTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTF
lcl|Query_10003               LGLECSPRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWNRKELLVTF
lcl|Query_10004               LTLDCEPRSGIDFNEMILMKMKKKTWLVHKQWFLDLPLPWTAGADTSEVHWNYKERMVTF
                                  * ** * ***** *  *  * * ** *** ******  ** *    *      ***

lcl|Query_10001               KTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTTTIFAGHLKCRLKMDKLTLKGMSYV
lcl|Query_10002               KNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGNLLFTGHLKCRLRMDKLQLKGMSYS
lcl|Query_10003               KNAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGTSIFAGHLKCRLKMDKLELKGMSYA
lcl|Query_10004               KVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDGNHMFAGHLKCKVRMEKLRIKGMSYT
                              *  *** * * ********** ** ****         * *****   * **  *****

lcl|Query_10001               MCTGSFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQDEKGVTQNGRLITANPIVTD
lcl|Query_10002               MCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIMDLEKRHVLGRLITVNPIVTE
lcl|Query_10003               MCTNTFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTEDGQGKAHNGRLITANPVVTK
lcl|Query_10004               MCSGKFSIDKEMAETQHGTTVVKVKYEGAGAPCKVPIEIRDVNKEKVVGRVISATPLAEN
                              **   *   **  ******      * *   *** *    *       ** *   *

lcl|Query_10001               KEKPVNIEAEPPFGESYIVIGAGEKALKLSWFKKGSSIGKMFEATARGARRMAILGDTAW
lcl|Query_10002               KDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRGAKRMAILGDTAW
lcl|Query_10003               KEEPVNIEAEPPFGESNIVIGIGDNALKINWYKKGSSIGKMFEATARGARRMAILGDTAW
lcl|Query_10004               TNSVTNIELEPPFGDSYIVIGVGNSALTLHWFRKGSSIGKMFESTYRGAKRMAILGETAW
                                   *** ***** * * **     *   *  ****** *** * *** ****** ***

lcl|Query_10001               DFGSIGGVFTSVGKLVHQIFGTAYGVLFSGVSWTMKIGIGVLLTWLGLNSRSTSLSMTCI
lcl|Query_10002               DFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGMNSRSTSLSVSLV
lcl|Query_10003               DFGSVGGVLNSLGKMVHQIFGSAYTALFSGVSWVMKIGIGVLLTWIGLNSKNTSMSFSCI
lcl|Query_10004               DFGSVGGLFTSLGKAVHQVFGSVYTTMFGGVSWMIRILIGFLVLWIGTNSRNTSMAMTCI
                              **** **   * **  ** **  *   * ****   * **    * * **  **

lcl|Query_10001               AVGLVTLYLGVMVQA
lcl|Query_10002               LVGVVTLYLGVMVQA
lcl|Query_10003               AIGIITLYLGAVVQA
lcl|Query_10004               AVGGITLFLGFTVQA
                                *  ** **  ***
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Figure 3: Multiple Sequence Alignment among the 4 Mosaics as Shown in Clustal Format (Papadopoulos & Agarwala, 2007)

Homology Detection

Submission of the mosaic sequences to HHpred server showed that the mosaic 1 protein is homologous to 42 protein while mosaic 2, 3 and 4 are homologous to 40, 42 and 41 proteins respectively including envelop protein of all four dengue serotype and other virus like zika, Japanese encephalitis, yellow fever, west nile, rift valley, herpes virus 1 etc. and non-structural helicase of some other virus including tick-borne encephalitis virus, JEV, DENV 2 and 3, Zika, Murray valley encephalitis, Kokobora among others. It also detected homology of the proteins with some antibody against dengue and other virus but, no human protein has been shown to be homologous (Zimmermann *et al.*, 2018). The homology chart of the mosaic proteins is demonstrated hereunder in figure-4.

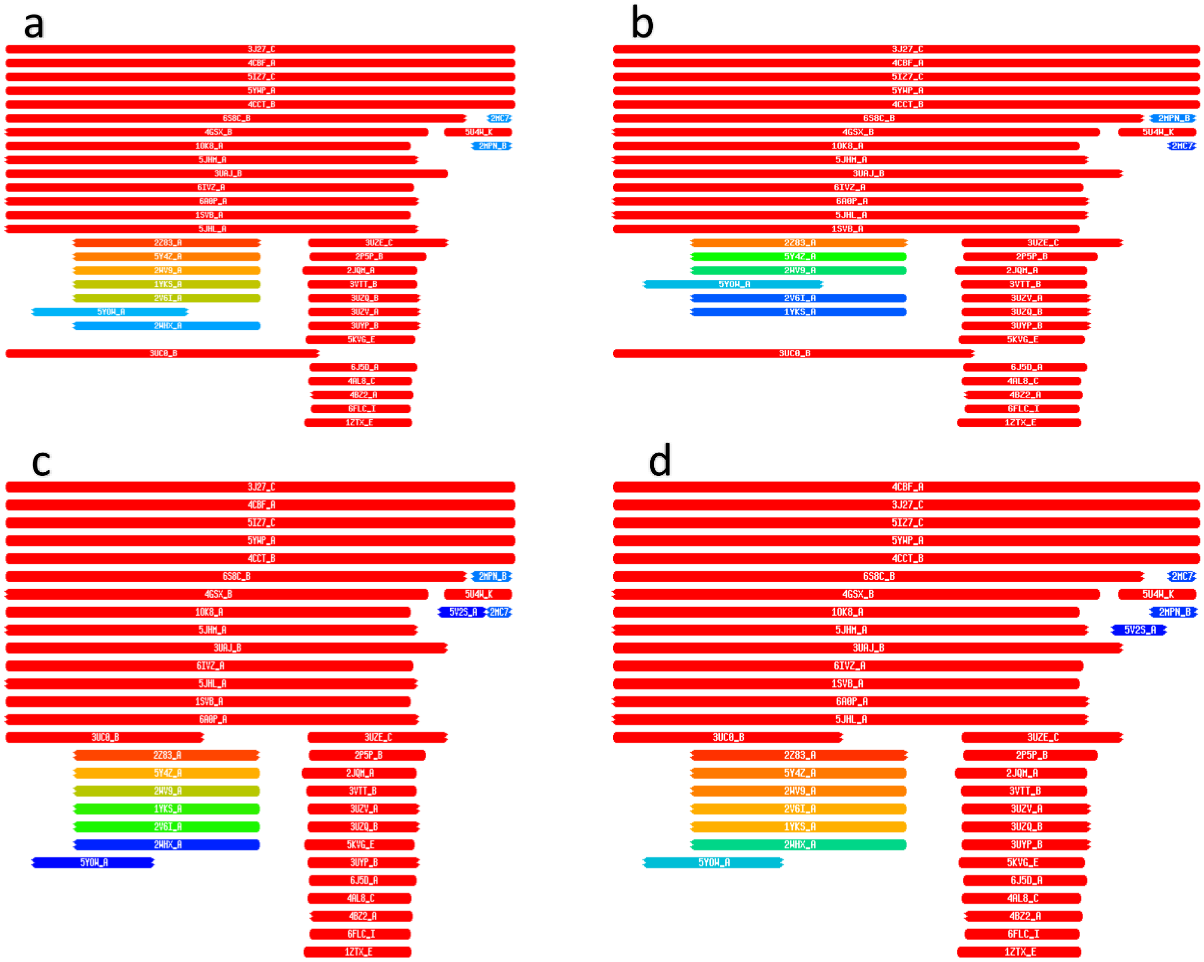

Figure 4: Homology Chart of Mosaic Proteins Detected by HHpred. a – Mosaic 1, b – Mosaic 2, c – Mosaic 3, d – Mosaic 4 (Zimmermann et al., 2018)

Protein Structure Prediction and Analysis

The length of the mosaic proteins is similar to that of original envelop proteins that is, DENV – 1, 2 and 4 are composed of 495 amino acids while that of DENV-3 is 493 amino acids. The proteins were first analysed for their secondary and tertiary structure by 2struc and IntFOLD server respectively.

Analysis of the mosaic 1 for its secondary structure revealed that it contains 16.8% α-helix(H), 36.4% β sheets(E), 3.2% 3_{10} helix(G), 13.7% bend(S) and 7.3% turn(T) and 21.8% loop and other irregular structure(-) (22, 23). The IntFOLD server predicted different tertiary structure with global model quality model score ranging from 0.4575 to 0.1978, where score more than 0.4 indicate good structure quality and stability and score less than 0.2 indicate poor model quality and incorrectly modelled structure. Both of the predicted top two models possessed same 0.4575 global model quality score, but the model tagged with 'IntFOLD5 COMA multi3 TS1' had lesser p value (5.468E-6) compared to the p value (5.481E-6) of the other one. So, this 'IntFOLD5 COMA multi3 TS1' protein was selected as our mosaic 1 protein model and named as 'Mos1TS1'. The local model quality score, as evaluated by the same server shows good per residue stability of the structure. DISOclust revealed a negligible disorder score (0.51) at residue 495 which is just above the cut-off value of 0.5. The predicted tertiary structure and local model quality plot as well as the disorder prediction of the model as carried out by DISOclust for Mos1TS1 is shown in figure-5 (19, 21).

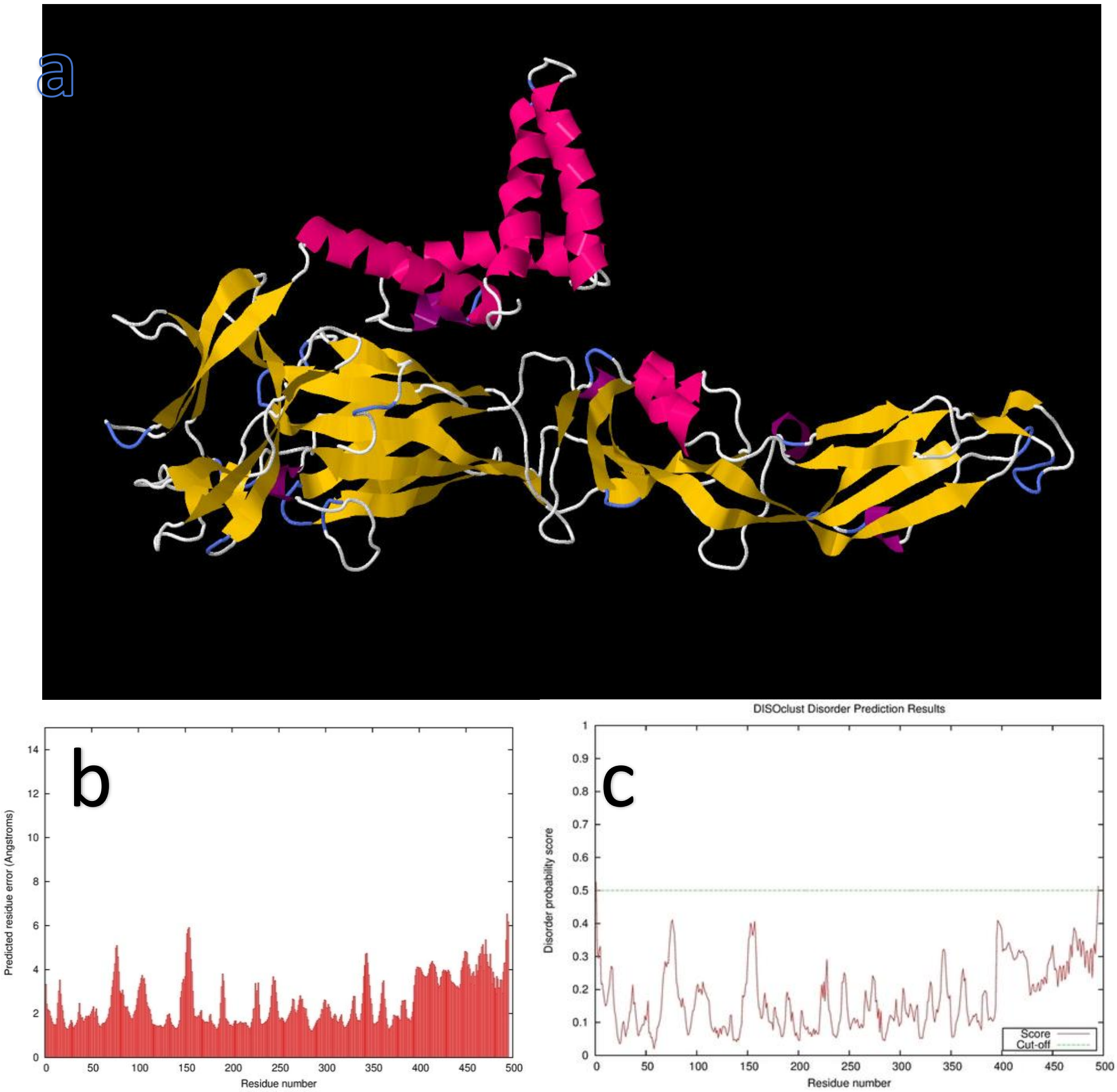

Figure 5:Tertiary Structure Prediction of Mos1TS1. a – Image View of Selected Tertiary Structure Model, b – Local Model Quality Plot to Asses the Model by Each Residue, c – Disorder Prediction Plot as Assessed by Disoclust (19, 21)

In case of mosaic 2 the proportion of secondary structures were 14.5% α-helix(H), 37.6% β sheets(E), 3.4% 3_{10} helix(G), 10.1% bend(S) and 12.9% turn(T) and 19.6% loop and other irregular structure(-) (22, 23). The top predicted model shows a good stable 3D structure model (figure-6a) that is blessed with global model quality score of 0.4375 and p value of 1.9E-6. The local model quality score depicts the chance of residue error only at terminal part of the chain specially in the 495th residue (figure-6b). The disorder prediction score as depicted by DISOclust is slightly higher (0.58) near residue 75 and at residue 495 (0.51) than the cut-off value (0.5) (figure-6c). With all these parameters setting it selected the model for further study and named it as ‘Mos2TS1’.

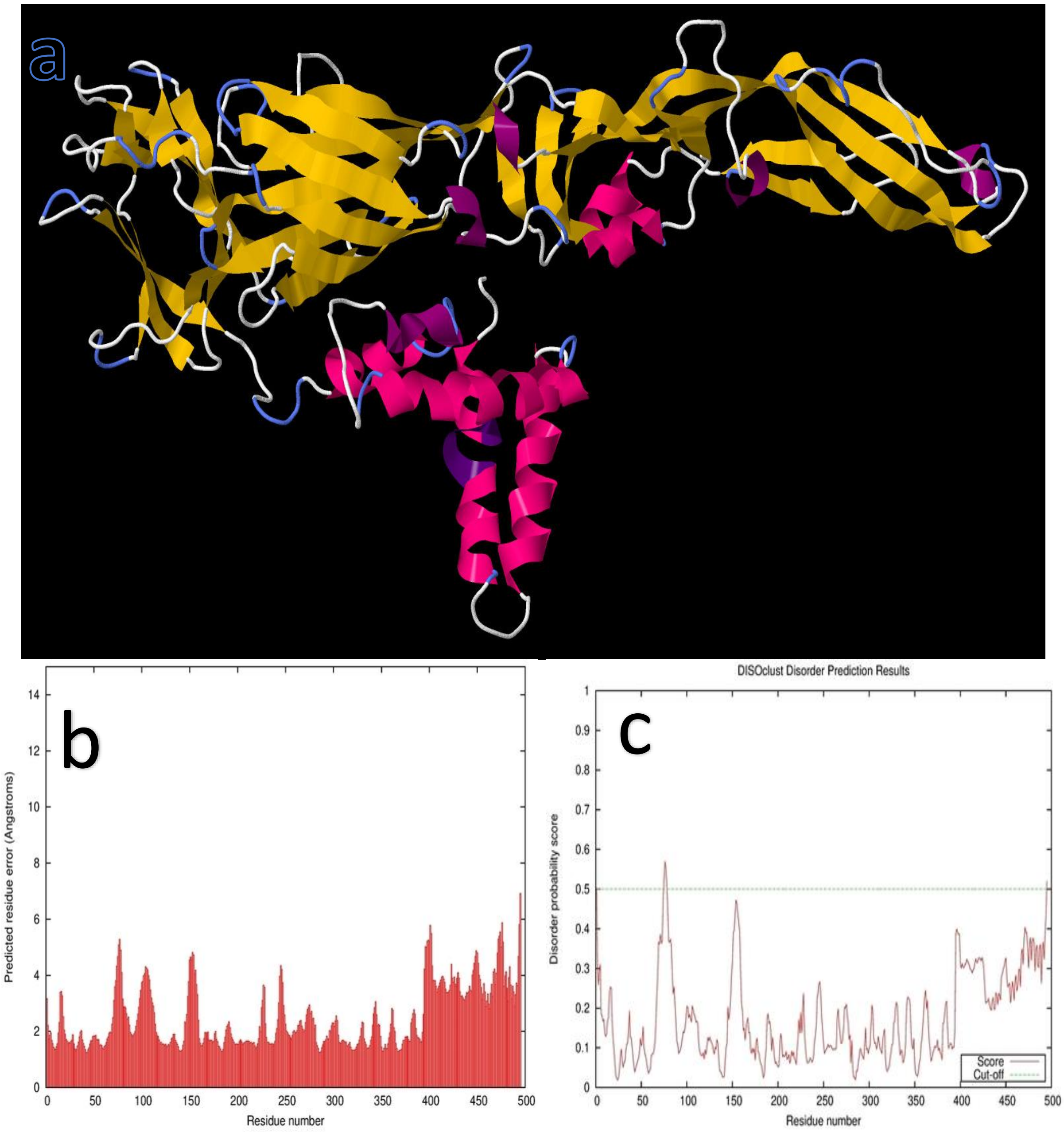

Figure 6:Tertiary Structure Prediction of Mos2TS1. a – Image View of Selected Tertiary Structure Model, b – Local Model Quality Plot to Assess the Model by Each Residue, c – Disorder Prediction Plot as Assessed by DISOclust (19, 21)

For mosaic 3 the composition of secondary structure is 17.8% α-helix(H), 36.3% β sheets(E), 2.6% 3_{10} helix(G), 12.2% bend(S) and 9.9% turn(T) and 19.7% loop and other irregular structure(-) (22, 23). The top predicted model tagged as 'IntFOLD5 IntFOLDTS140 multi3 TS1' shows a stable 3D structure model (figure-7a) with global model quality score of 0.4705 and p value of 2.314E-6. The local model quality score depicts the probability of residue error more than 6 angstrom only at terminal end of the chain in the 493rd residue (figure-7b). The disorder prediction score as depicted by DISOclust is slightly higher at terminal residue 493 (0.51) than the cut-off value (0.5) (figure-7c). Following all these evaluations it selected the model for further study and named it as 'Mos3TS1'.

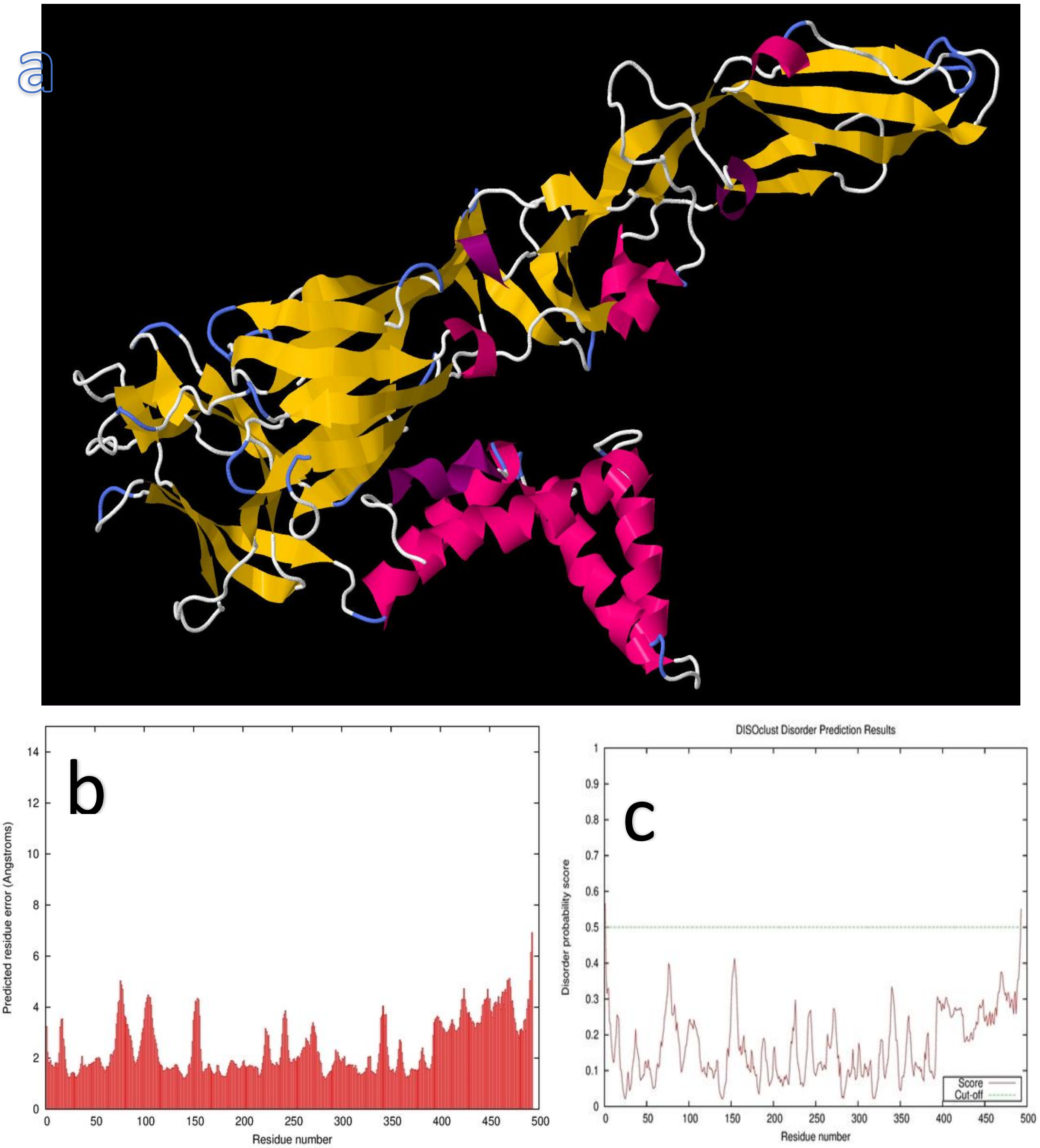

Figure 7: Tertiary structure prediction of Mos3TS1. a – image view of selected tertiary structure model, b – local model quality plot to assess the model by each residue, c – disorder prediction plot as assessed by DISOclust (19, 21)

The secondary structure distribution of mosaic 4 is 17.2% α-helix(H), 37.4% β sheets(E), 3.0% 3_{10} helix(G), 12.3% bend(S) and 10.1% turn(T) and 19.0% loop and other irregular structure(-) (22, 23). The top predicted model tagged as 'IntFOLD5 IntFOLDTS60 multi3 TS1' depicts a good 3D structure model (figure-8a) with global model quality score of 0.4726 and p value of 2.021E-6. The local model quality score predicts the probability of residue error more than 6 angstrom only at terminal end of the chain in the 494th and 495th residue (figure-8b). The disorder prediction scores as depicted by DISOclust is slightly higher at terminal residue 495 (0.54) than the cut-off value (0.5) (figure-8c). As it satisfies the criteria the model was selected for further study and was named as 'Mos4TS1'.

Discussion

Dengue virus continues to pose a significant global health threat, with hundreds of millions infected annually and a substantial burden of severe disease (Liu, 2025). The limitations of the only currently licensed vaccine, Dengvaxia, particularly its variable efficacy and increased risk of severe dengue in seronegative individuals, underscore the urgent need for novel vaccine strategies (Norshidah *et al*., 2021; See, 2025; Torres-Flores *et al*., 2022; Yong *et al*., 2022). A critical challenge in dengue vaccine development is Antibody-Dependent Enhancement, which necessitates vaccine designs that elicit balanced immunity across all four serotypes to avoid exacerbating disease severity (Chen *et al*., 2023; Izmirly *et al*., 2020; Liu, 2025). This study addresses these challenges by employing a bioinformatic approach to design a mosaic vaccine strategy for the DENV envelope (E) protein, aiming for a stronger, broader immune response with an enhanced safety profile.

The results strongly suggest that a mosaic E protein construct is a potential candidate as a dengue vaccine. The production of new mosaic E proteins based on conserved sequences for several serotypes has demonstrated the potential to encompass a deeper antigenic diversity and assure broad protection. High sequence identity (96-99%) of the designed mosaic proteins with their wild-type E protein counterparts would also make the elicited immune response relevant to natural DENV infection. This relatively high degree of conservation, with not a lot of gaps in the alignment supports the fact that these mosaic proteins still harbor important antigenic determinants, as well preserves epitopes from multiple serotypes for broad coverage (Kaushik *et al*., 2022).

Findings of the structural predictions provide another evidence to support the authenticity of the mosaic model. The predicted secondary structure, highly enriched in β sheets, agrees with the flexibility that is known for the native E protein and that it is essential to recognize the virus by an immune system. Importantly, the top-score predicted 3D models of each serotype showed good global model quality scores (0.4375-0.4726), demonstrating stable and biologically relevant three-dimensional structures (Figures 5-7). The little disorder that was observed, especially at the terminals, would seem to indicate that these mosaic proteins are expected to be structural intact which is important for efficient presentation of epitopes to the immune system (Medits *et al*., 2021; Roy, 2020). Such bioinformatic structural analyses are important, as preservation of the correct conformations of epitopes is key for the induction of neutralizing antibodies and T cell responses against a highly variable virus such as dengue (Shanshin *et al*., 2022).

Finally, the immunoinformatic analysis demonstrated that the mosaic proteins exhibited similar or superior antigenicity and immunogenicity compared to the wild-type E proteins. This in silico prediction of strong B-cell and T-cell epitopes suggests that the mosaic design could effectively prime both humoral and cellular immunity. This finding is consistent with recent advancements in immunoinformatics and reverse vaccinology, which have proven to be powerful tools for designing multi-epitope vaccines against various pathogens, including DENV (Dixit, 2021; Fadaka *et al*., 2021; Kaushik *et al*., 2022). These computational approaches facilitate the identification of immunodominant epitopes and the engineering of constructs that can overcome the challenges of DENV antigenic diversity and the need for broad, serotype-balanced protection (Biner *et al*., 2020; Dixit, 2021; Fadaka *et al*., 2021; Mahata *et al*., 2021). The success of this in silico design provides a compelling argument for its potential utility, supporting the development of a vaccine that represents the antigenic diversity of thousands of proteins within a single, manageable construct. Novel vaccine platforms, such as those employing computationally optimized broadly reactive antigen algorithms, have shown promise in eliciting broadly neutralizing antibodies against all four serotypes, regardless of prior DENV exposure, further supporting the strategic direction of the uamosaic design (Liu, 2025).

In conclusion, this bioinformatic analysis provides strong evidence for the potential of a mosaic E protein-based vaccine to overcome the limitations of existing dengue vaccines. The designed mosaic proteins demonstrate high similarity to native proteins, maintain favorable structural characteristics, exhibit no homology to human proteins, and predict strong antigenicity and immunogenicity. While these results are highly encouraging, further experimental validation through in vitro and in vivo studies will be crucial to confirm the immunogenicity, safety, and cross-protective efficacy of these mosaic constructs against all four DENV serotypes. This work represents a significant step towards the development of a safe and broadly effective dengue vaccine.

Limitation

This study is limited by its reliance on silico bioinformatic and immunoinformatic analyses without experimental validation. The findings require confirmation through **in vitro and in vivo studies** to assess real immune responses, safety, and antibody-dependent enhancement risk. In addition, the analysis focuses only on the dengue virus envelope protein and may not fully capture the contribution of other viral antigens or global strain diversity.

Conclusion

This study presents a comprehensive bioinformatic and immunoinformatic evaluation of a mosaic dengue vaccine strategy based on the envelope (E) protein. The designed mosaic proteins demonstrated high sequence identity (96–99%) with native dengue virus E proteins across all four serotypes, preserved key structural features, and showed favorable antigenicity and immunogenicity profiles in silico.

Structural modeling confirmed that the mosaic constructs maintain biologically relevant secondary and tertiary conformations, with acceptable global and local model quality scores and minimal structural disorder. Importantly, homology analysis revealed no similarity to human proteins, supporting the potential safety of the proposed vaccine design.

Overall, the findings suggest that a mosaic E protein-based vaccine could represent conserved antigenic diversity across dengue virus serotypes within a single construct, offering a promising solution to challenges such as serotype imbalance and antibody-dependent enhancement seen with existing vaccines.

However, these conclusions are preliminary and require rigorous experimental validation, including laboratory-based expression studies, animal model testing, immunogenicity assays, and ADE evaluation. If validated, this mosaic vaccine strategy may contribute significantly to the development of a safe, broadly protective, next-generation dengue vaccine.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Acknowledgement

The authors would like to acknowledge all researchers and institutions whose publicly available databases, tools, and resources made this study possible.

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