**Supplementary Tables**

**Article title**

**Targeting “Immunogenic Hotspots” in Dengue and Zika Virus:**

**An In-silico Approach to a Common Vaccine Candidate**

**Table S1: Antigenic probability of the structural proteins as determined by the VaxiJen server.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Proteins** | **Antigenic Probability** | | | | | **Average Probability** |
|  | **DENV1** | **DENV2** | **DENV3** | **DENV4** | **ZIKV** |  |
| **Anchored Capsid Protein (AncC)** | **0.4003** | **0.3877** | **0.4335** | **0.2336** | **0.4677** | **0.3846** |
| **Capsid Protein (C)** | **0.3778** | **0.3693** | **0.3641** | **0.1636** | **0.3856** | **0.332** |
| **Membrane Glycoprotein Precursor (PreM)** | **0.6391** | **0.5796** | **0.6585** | **0.6273** | **0.541** | **0.6091** |
| **Membrane Glycoprotein (M)** | **0.6168** | **0.502** | **0.5769** | **0.5101** | **0.38** | **0.5172** |
| **Envelope Protein (E)** | **0.7495** | **0.6695** | **0.7367** | **0.6442** | **0.6201** | **0.684** |

**Table S2:** Antigenic probabilities of the non-structural proteins as determined by the VaxiJen server

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Proteins** | **Antigenic Probability** | | | | | **Average Probability** |
| **DENV1** | **DENV2** | **DENV3** | **DENV4** | **ZIKV** |
| Non Structural Protein 1 (NS1) | 0.4955 | 0.607 | 0.4879 | 0.515 | 0.4521 | 0.5115 |
| Non Structural Protein 2A (NS2A) | 0.718 | 0.7747 | 0.7198 | 0.5361 | 0.6549 | 0.6807 |
| Non Structural Protein 2B (NS2B) | 0.571 | 0.6959 | 0.4411 | 0.6721 | 0.5526 | 0.58654 |
| Non Structural Protein 3 (NS3) | 0.5146 | 0.5762 | 0.5496 | 0.5904 | 0.4886 | 0.54388 |
| Non Structural Protein 4A (NS4A) | 0.413 | 0.4465 | 0.4516 | 0.4525 | 0.5977 | 0.47226 |
| Non Structural Protein 4B (NS4B) | 0.6415 | 0.6135 | 0.5581 | 0.5984 | 0.5856 | 0.59942 |
| Non Structural Protein 5 (NS5) | 0.4394 | 0.4427 | 0.4533 | 0.4358 | 0.4332 | 0.44088 |

**Table S3:** Linear B cell immunogenic hotspots identified from PreM, E and NS5 proteins of all DENV serotypes and ZIKV. The stretches in bold depict common regions to MHC class I and MHC class II restricted immunogenic hotspot.

|  |  |  |  |
| --- | --- | --- | --- |
| **Virus** | **Protein** | **Position** | **Number of predicted linear B cell epitopes** |
| DENV1 | PreM | 42-122 | 22 |
| DENV2 | 10-156 | 27 |
| DENV3 | 33-156 | 23 |
| DENV4 | 34-138 | 21 |
| ZIKV | 2-98 | 23 |
| DENV1 | E | 257-392 | 19 |
| DENV2 | 317-390 | 11 |
| DENV3 | 272-384 | 13 |
| DENV4 | 257-389 | 19 |
| ZIKV | 282-389 | 19 |
| DENV1 | NS5 | 81-127  283-325  **434-496**  **514-554**  621-706  **711-760**  760-862 | 11  9  6  6  13  6  18 |
| DENV2 | 69-128  282-326  **444-497**  **508-550**  618-707  **727-866** | 16  10  5  6  13  34 |
| DENV3 | 69-127  261-322  **445-513**  **518-566**  620-707  **712-782**  782-863 | 13  10  8  9  14  10  16 |
| DENV4 | 79-126  282-326  **444-505**  **513-579**  617-708  **728-777**  783-867 | 14  13  7  8  14  7  17 |
| ZIKV | 79-128  285-327  **437-515**  **518-581**  624-713  **720-784**  784-870 | 10  9  7  12  12  10  15 |

**Table S4**: Number of contact residues for thee predicted epitope (101-103) and the rest of the fusion peptide loop region (97-111) of E protein, as predicted by Discotope 2.0 (PDB Id for each protein is put in bracket)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Position** | **Residue** | **Contact number** | | | |
| **ZIKV (5IRE)** | **DENV2 (3C5X)** | **DENV3 (1UZG)** | **DENV4 (3UAJ)** |
| 98 | ASP | No epitope predicted | 4 | No epitope predicted | No epitope predicted |
| 100 | GLY | 6 | 4 | 4 |
| 101 | TRP | 1 | 0 | 0 | No epitope predicted |
| 102 | GLY | 0 | 8 | 9 | 5 |
| 103 | ASN | No epitope predicted | 20 | No epitope predicted | No epitope predicted |
| 104 | GLY | 12 |
| 106 | GLY | 2 | 1 |
| 107 | LEU | 10 | No epitope predicted |
| 108 | PHE | 1 | 2 |
| 109 | GLY | 12 | No epitope predicted |
| 110 | LYS | 8 |

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**Table S5:** Number of contact residues of the conserved epitopes from NS5 protein as predicted by Discotope 2.0(PDB Id for each protein is put in bracket).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Position of the conserved residues within predicted epitopes** | **Residue** | **Contact numbers** | | |
| **DENV2 (5ZQK)** | **DENV3 (5DTO)** | **ZIKV (5U0B)** |
| Conserved residues within 14-48 region  23  24  27  28  29  30  31 | GLU  PHE  TYR  LYS  LYS  SER  GLY | 11  13  23  11  13  21  15 | 9  13  24  10  13  21  18 | 14  12  24  9  9  21  16 |
| Conserved residues of 107-109 region  107  108  109 | GLY  PRO  GLY | 5  0  2 | 8  0  3 | 7  3  2 |
| Conserved residues within 303-318 region  303  304  305  310  311 | THR  TRP  ALA  TYR  GLU | 16  31  20  16  6 | 23  33  20  15  6 | 18  33  20  17  6 |
| Conserved residues within 355-363 region  355  356  357  358  359  360  361  362  363 | VAL  PHE  LYS  GLU  LYS  VAL  ASP  THR  ARG | 30  37  29  18  25  31  25  22  12 | 18  34  30  24  19  25  32  31  23 | 27  35  32  20  23  31  28  23  14 |
| Conserved residues within 719-725 region  721  722  723  724 | LYS  ASP  GLY  ARG | 12  16  2  20 | 11  22  3  20 | 11  9  11  20 |
| Conserved residues within 817-834 region  828  829  830  831 | ASP  LYS  THR  PRO | 11  5  12  11 | 11  7  12  7 | 11  7  10  7 |

**Table S6:** Alignment result between the known structure of CTxB and the predicted MEV models. The top five candidates are in bold

|  |  |  |  |
| --- | --- | --- | --- |
| **Model** | **Combination** | **No. of Cα atom aligned** | **RMSD (Å)** |
| Model 1 | ABCD | 98 | 17.057 |
| Model 2 | ABDC | 99 | 16.029 |
| Model 3 | ADBC | 82 | 0.565 |
| Model 4 | ADCB | 76 | 4.319 |
| Model 5 | ACDB | 79 | 0.680 |
| Model 6 | ACBD | 98 | 16.550 |
| Model 7 | BCDA | 80 | 0.349 |
| **Model 8** | **BCAD** | **85** | **0.398** |
| **Model 9** | **BACD** | **85** | **0.413** |
| Model 10 | BADC | 89 | 1.190 |
| Model 11 | BDAC | 80 | 0.364 |
| Model 12 | BDCA | 82 | 0.413 |
| **Model 13** | **CDAB** | **83** | **0.462** |
| Model 14 | CDBA | 80 | 0.386 |
| Model 15 | CBDA | 76 | 0.444 |
| Model 16 | CBAD | 95 | 13.101 |
| Model 17 | CADB | 78 | 0.497 |
| Model 18 | CABD | 76 | 0.401 |
| **Model 19** | **DABC** | **84** | **0.409** |
| Model 20 | DACB | 82 | 0.411 |
| **Model 21** | **DBCA** | **84** | **0.408** |
| Model 22 | DBAC | 82 | 0.493 |
| Model 23 | DCBA | 81 | 0.381 |
| Model 24 | DCAB | 78 | 0.390 |

**Table S7:** Gibbs free energy for folding of the selected models

|  |  |
| --- | --- |
| **Model** | **Gibbs free energy (Kcal/mol)** |
| Model 8 | 91.37 |
| Model 9 | 83.25 |
| Model 13 | 109.80 |
| Model 19 | 85.43 |
| Model 21 | 87.19 |

**Table S8:** Conserved Epitopes for DENV serotypes and ZIKV as obtained from

the MEV models.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **MEV Model** | **Number of Conserved Epitopes for DENV serotypes and ZIKV** | | | | | |
| **MHC class I Epitopes** | | **MHC Class II Epitopes** | | **Linear B Cell Epitopes** | |
| Model 8 | 16 | All previously predicted epitopes are conserved | 15 | All previously predicted epitopes are conserved | 2 | All previously predicted epitopes are conserved |
| Model 9 | 16 | 15 | 2 |
| Model 19 | 16 | 15 | 2 |
| Model 21 | 16 | 15 | 2 |

**Table S9: IFN-γ inducing epitopes predicted from the MEV models using IFNepitope server. Epitopes having a score greater than 0.8 were considered as high scoring epitopes.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **MEV** | **Start-End** | **Sequence** | **Method** | **Score** | **Part of the MEV** |
| **All** | **92-109** | [**PHAIAAISMANEAAAKC**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=PHAIAAISMANEAAAKC&method=hybrid&model=main) | **SVM** | **1.2698526** | **Adjuvant** |
| **93-110** | [**HAIAAISMANEAAAKCV**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=HAIAAISMANEAAAKCV&method=hybrid&model=main) | **SVM** | **1.2679528** |
| **0-17** | [**TPQNITDLCAEYHNTQI**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=TPQNITDLCAEYHNTQI&method=hybrid&model=main) | **MERCI** | **1** |
| **1-18** | [**PQNITDLCAEYHNTQIH**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=PQNITDLCAEYHNTQIH&method=hybrid&model=main) | **MERCI** | **1** |
| **2-19** | [**QNITDLCAEYHNTQIHT**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=QNITDLCAEYHNTQIHT&method=hybrid&model=main) | **MERCI** | **1** |
| **3-20** | [**NITDLCAEYHNTQIHTL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=NITDLCAEYHNTQIHTL&method=hybrid&model=main) | **MERCI** | **1** |
| **18-35** | [**TLNDKIFSYTESLAGKR**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=TLNDKIFSYTESLAGKR&method=hybrid&model=main) | **MERCI** | **1** |
| **19-36** | [**LNDKIFSYTESLAGKRE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=LNDKIFSYTESLAGKRE&method=hybrid&model=main) | **MERCI** | **1** |
| **20-37** | [**NDKIFSYTESLAGKREM**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=NDKIFSYTESLAGKREM&method=hybrid&model=main) | **MERCI** | **1** |
| **21-38** | [**DKIFSYTESLAGKREMA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=DKIFSYTESLAGKREMA&method=hybrid&model=main) | **MERCI** | **1** |
| **24-41** | [**FSYTESLAGKREMAIIT**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=FSYTESLAGKREMAIIT&method=hybrid&model=main) | **MERCI** | **1** |
| **25-42** | [**SYTESLAGKREMAIITF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=SYTESLAGKREMAIITF&method=hybrid&model=main) | **MERCI** | **1** |
| **26-43** | [**YTESLAGKREMAIITFK**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=YTESLAGKREMAIITFK&method=hybrid&model=main) | **MERCI** | **1** |
| **MEV 8** | **103-120** | [**EAAAKCVYNMMGKREKK**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=EAAAKCVYNMMGKREKK&method=hybrid&model=main) | **SVM** | **1.0941695** | **B hotspot** |
| **104-121** | [**AAAKCVYNMMGKREKKG**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AAAKCVYNMMGKREKKG&method=hybrid&model=main) | **SVM** | **0.98919999** |
| **150-167** | [**RAIWYMWLGARFLEFEA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=RAIWYMWLGARFLEFEA&method=hybrid&model=main) | **SVM** | **1.1849434** | **A hotspot** |
| **145-162** | [**GAKGSRAIWYMWLGARF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GAKGSRAIWYMWLGARF&method=hybrid&model=main) | **SVM** | **1.1701188** |
| **144-161** | [**PGAKGSRAIWYMWLGAR**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=PGAKGSRAIWYMWLGAR&method=hybrid&model=main) | **SVM** | **1.150083** |
| **149-166** | [**SRAIWYMWLGARFLEFE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=SRAIWYMWLGARFLEFE&method=hybrid&model=main) | **SVM** | **1.1349337** |
| **147-164** | [**KGSRAIWYMWLGARFLE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=KGSRAIWYMWLGARFLE&method=hybrid&model=main) | **SVM** | **1.1160535** |
| **148-165** | [**GSRAIWYMWLGARFLEF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GSRAIWYMWLGARFLEF&method=hybrid&model=main) | **SVM** | **1.1002294** |
| **146-163** | [**AKGSRAIWYMWLGARFL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AKGSRAIWYMWLGARFL&method=hybrid&model=main) | **SVM** | **1.0320195** |
| **143-160** | [**GPGAKGSRAIWYMWLGA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GPGAKGSRAIWYMWLGA&method=hybrid&model=main) | **SVM** | **0.90672798** |
| **152-169** | [**IWYMWLGARFLEFEALG**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=IWYMWLGARFLEFEALG&method=hybrid&model=main) | **SVM** | **0.90228323** |
| **151-168** | [**AIWYMWLGARFLEFEAL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AIWYMWLGARFLEFEAL&method=hybrid&model=main) | **SVM** | **0.87490682** |
| **MEV 9** | **103-120** | [**EAAAKCVYNMMGKREKK**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=EAAAKCVYNMMGKREKK&method=hybrid&model=main) | **SVM** | **1.0941695** | **B hotspot** |
| **104-121** | [**AAAKCVYNMMGKREKKG**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AAAKCVYNMMGKREKKG&method=hybrid&model=main) | **SVM** | **0.98919999** |
| **131-148** | [**RAIWYMWLGARFLEFEA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=RAIWYMWLGARFLEFEA&method=hybrid&model=main) | **SVM** | **1.1849434** | **A hotspot** |
| **126-143** | [**GAKGSRAIWYMWLGARF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GAKGSRAIWYMWLGARF&method=hybrid&model=main) | **SVM** | **1.1701188** |
| **125-142** | [**PGAKGSRAIWYMWLGAR**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=PGAKGSRAIWYMWLGAR&method=hybrid&model=main) | **SVM** | **1.150083** |
| **130-147** | [**SRAIWYMWLGARFLEFE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=SRAIWYMWLGARFLEFE&method=hybrid&model=main) | **SVM** | **1.1349337** |
| **128-145** | [**KGSRAIWYMWLGARFLE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=KGSRAIWYMWLGARFLE&method=hybrid&model=main) | **SVM** | **1.1160535** |
| **129-146** | [**GSRAIWYMWLGARFLEF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GSRAIWYMWLGARFLEF&method=hybrid&model=main) | **SVM** | **1.1002294** |
| **127-144** | [**AKGSRAIWYMWLGARFL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AKGSRAIWYMWLGARFL&method=hybrid&model=main) | **SVM** | **1.0320195** |
| **124-141** | [**GPGAKGSRAIWYMWLGA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GPGAKGSRAIWYMWLGA&method=hybrid&model=main) | **SVM** | **0.90672798** |
| **133-150** | [**IWYMWLGARFLEFEALG**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=IWYMWLGARFLEFEALG&method=hybrid&model=main) | **SVM** | **0.90228323** |
| **132-149** | [**AIWYMWLGARFLEFEAL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AIWYMWLGARFLEFEAL&method=hybrid&model=main) | **SVM** | **0.87490682** |
| **MEV 19** | **134-151** | [**RAIWYMWLGARFLEFEA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=RAIWYMWLGARFLEFEA&method=hybrid&model=main) | **SVM** | **1.1849434** | **A hotspot** |
| **129-146** | [**GAKGSRAIWYMWLGARF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GAKGSRAIWYMWLGARF&method=hybrid&model=main) | **SVM** | **1.1701188** |
| **128-145** | [**PGAKGSRAIWYMWLGAR**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=PGAKGSRAIWYMWLGAR&method=hybrid&model=main) | **SVM** | **1.150083** |
| **133-150** | [**SRAIWYMWLGARFLEFE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=SRAIWYMWLGARFLEFE&method=hybrid&model=main) | **SVM** | **1.1349337** |
| **131-148** | [**KGSRAIWYMWLGARFLE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=KGSRAIWYMWLGARFLE&method=hybrid&model=main) | **SVM** | **1.1160535** |
| **132-149** | [**GSRAIWYMWLGARFLEF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GSRAIWYMWLGARFLEF&method=hybrid&model=main) | **SVM** | **1.1002294** |
| **130-147** | [**AKGSRAIWYMWLGARFL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AKGSRAIWYMWLGARFL&method=hybrid&model=main) | **SVM** | **1.0320195** |
| **127-144** | [**GPGAKGSRAIWYMWLGA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GPGAKGSRAIWYMWLGA&method=hybrid&model=main) | **SVM** | **0.90672798** |
| **136-153** | [**IWYMWLGARFLEFEALG**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=IWYMWLGARFLEFEALG&method=hybrid&model=main) | **SVM** | **0.90228323** |
| **135-152** | [**AIWYMWLGARFLEFEAL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AIWYMWLGARFLEFEAL&method=hybrid&model=main) | **SVM** | **0.87490682** |
| **MEV 21** | **172-189** | [**RAIWYMWLGARFLEFEA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=RAIWYMWLGARFLEFEA&method=hybrid&model=main) | **SVM** | **1.1849434** | **A hotspot** |
| **167-184** | [**GAKGSRAIWYMWLGARF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GAKGSRAIWYMWLGARF&method=hybrid&model=main) | **SVM** | **1.1701188** |
| **166-183** | [**PGAKGSRAIWYMWLGAR**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=PGAKGSRAIWYMWLGAR&method=hybrid&model=main) | **SVM** | **1.150083** |
| **171-188** | [**SRAIWYMWLGARFLEFE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=SRAIWYMWLGARFLEFE&method=hybrid&model=main) | **SVM** | **1.1349337** |
| **169-186** | [**KGSRAIWYMWLGARFLE**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=KGSRAIWYMWLGARFLE&method=hybrid&model=main) | **SVM** | **1.1160535** |
| **170-187** | [**GSRAIWYMWLGARFLEF**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GSRAIWYMWLGARFLEF&method=hybrid&model=main) | **SVM** | **1.1002294** |
| **168-185** | [**AKGSRAIWYMWLGARFL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AKGSRAIWYMWLGARFL&method=hybrid&model=main) | **SVM** | **1.0320195** |
| **165-182** | [**GPGAKGSRAIWYMWLGA**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=GPGAKGSRAIWYMWLGA&method=hybrid&model=main) | **SVM** | **0.90672798** |
| **174-191** | [**IWYMWLGARFLEFEALG**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=IWYMWLGARFLEFEALG&method=hybrid&model=main) | **SVM** | **0.90228323** |
| **173-190** | [**AIWYMWLGARFLEFEAL**](http://crdd.osdd.net/raghava/ifnepitope/pep_design.php?sequence=AIWYMWLGARFLEFEAL&method=hybrid&model=main) | **SVM** | **0.87490682** |