# Supporting Information

# An Immunoinformatics Approach to Design a Potent Multi-Epitope Vaccine Against Asia-1 Genotype of Crimean-Congo Haemorrhagic Fever Virus Using the Structural Glycoproteins as a Target

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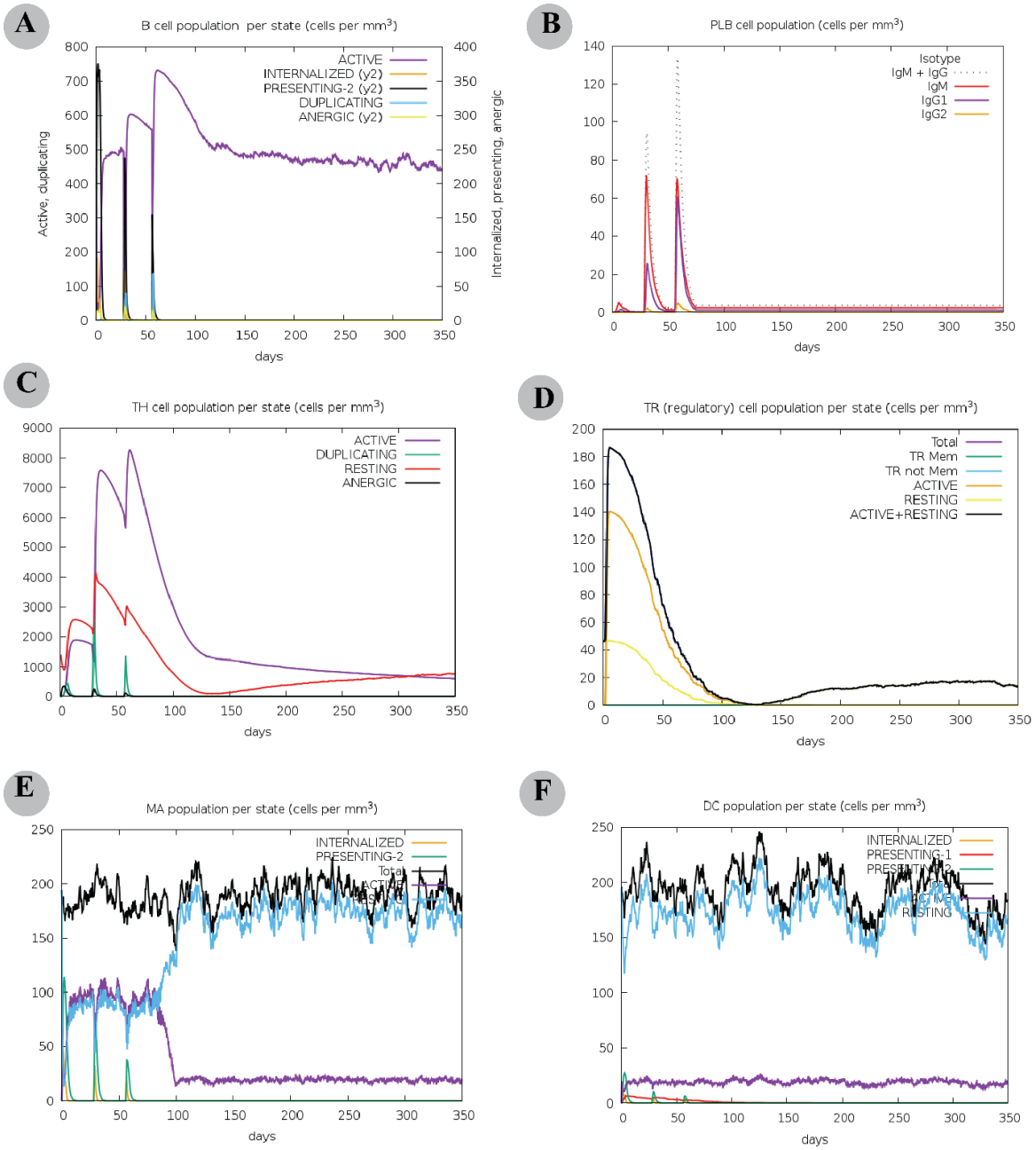


**Figure S1.** *In silico* immune simulation of an infection challenge, comprised of a virus responding to the sequence of Asia-1 genotype of CCHFV proteins covered by the multi-epitope construct, was simulated on day 350. **(A)** B lymphocytes population per entity-state (i.e., showing counts for active, presenting on class II, internalized the Ag, duplicating and anergic **(B)** Plasma B lymphocytes count sub-divided per isotype (IgM, IgG1 and IgG2) **(C)** CD4+ T-helper (TH) lymphocytes in several forms, i.e., active, duplicating (in the mitotic cycle), resting (not active), and anergic **(D)** CD4 T-regulatory lymphocytes count. Both total, memory and per entity-state counts are plotted here **(E)** Total count, internalized, presenting on MHC class-II, active and resting macrophages. **(F)** Dendritic cells can present antigenic peptides on both MHC class-I and class-II molecules. The curves show the total number broken down to active, resting, internalized, and presenting the ag.

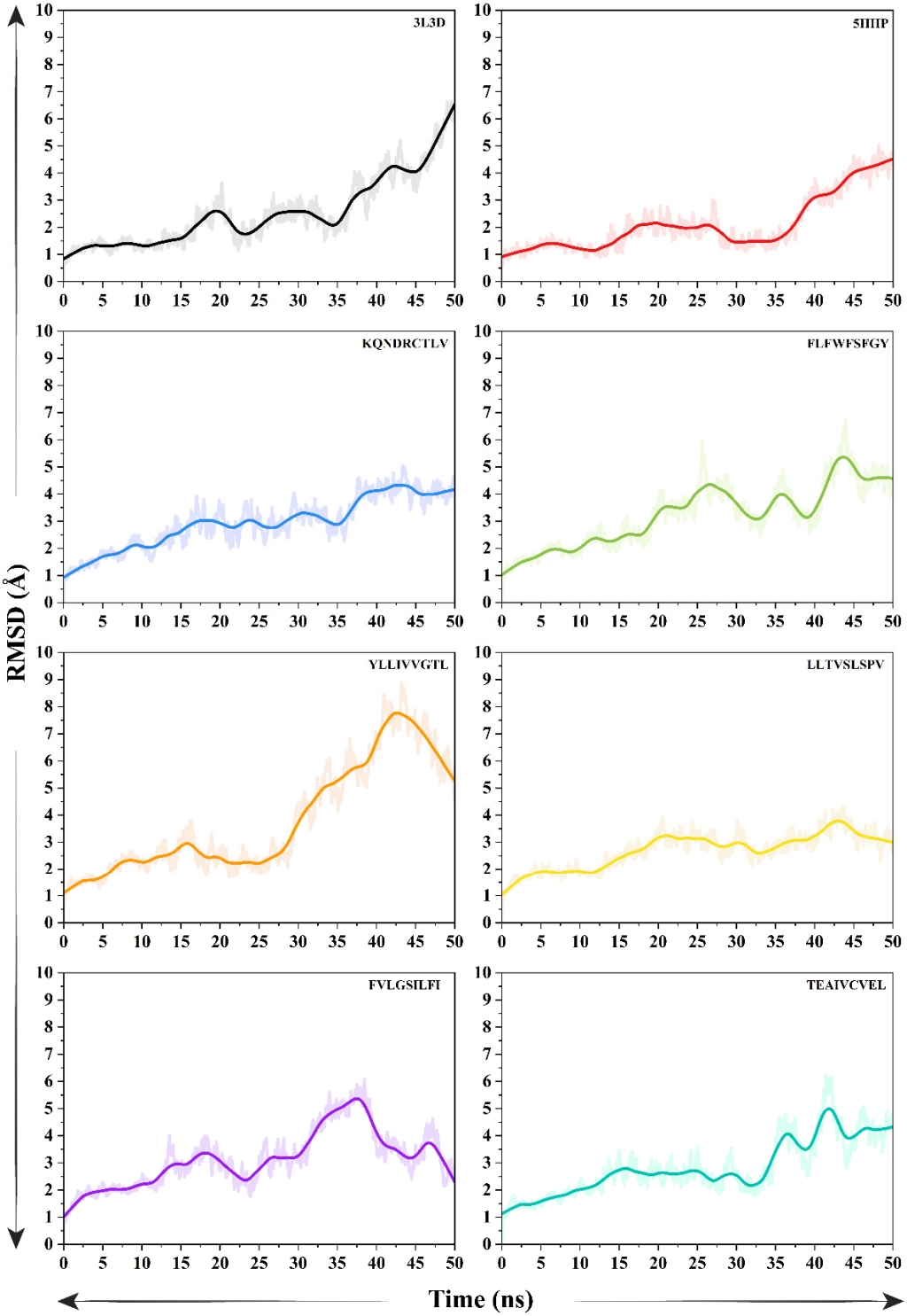


**Figure S2**. Root Mean Square Deviation (RMSD) analysis of the selected epitopes (KQNDRCTLV, FLFWFSFGY, YLLIVVGTL, LLTVSLSPV, FVLGSILFI) docked with the HLA-A*0201 molecule and epitope TEAIVCVEL docked with HLA-B*4402 molecule. The 3L3D (EEAGRAFSF and HLA-B*4402 complex) and 5HHP (GILEFVFTL and HLA-A*0201 complex) were selected as reference systems.

**Table S1.** Population coverage calculation result

| | Class I | | |
|---|---|---|---|
| population/area | coverage[a] | average hit[b] | pc90[c] |
| East Asia | 35.99% | 1.43 | 0.16 |
| Northeast Asia | 30.33% | 1.5 | 0.14 |
| Pakistan | 49.85% | 1.56 | 0.71 |
| South Asia | 31.98% | 1.44 | 0.15 |
| Northeast Asia | 29.51% | 1.44 | 0.14 |
| Southeast Asia | 45.54% | 2.28 | 0.18 |
| World | 60.52% | 3.07 | 0.25 |
| [a]projected population coverage | | | |
| [b]average number of epitope hits / HLA combinations recognized by the population | | | |
| [c]minimum number of epitope hits / HLA combinations recognized by 90% of the population | | | |

**Table S2.** GalaxyRefine output for the submitted initial model of vaccine constructs predicted by the I-TASSER server. (Letter in bold indicates the final model selected for further analysis).

| Model | GDT-HA | RMSD | MolProbity | Clash score | Poor rotamers | Rama favored |
|---|---|---|---|---|---|---|
| Initial | 1.0000 | 0.000 | 3.227 | 9.7 | 13.2 | 66.8 |
| MODEL 1 | 0.9275 | 0.469 | 2.423 | 20.6 | 0.8 | 87.3 |
| MODEL 2 | 0.9360 | 0.471 | 2.494 | 25.4 | 0.8 | 87.9 |
| **MODEL 3** | 0.9267 | 0.463 | 2.501 | 24.5 | 0.8 | 87.0 |
| MODEL 4 | 0.9174 | 0.490 | 2.498 | 24.7 | 0.8 | 87.3 |
| MODEL 5 | 0.9282 | 0.471 | 2.438 | 20.4 | 0.8 | 86.3 |

**Table S3.** Docking score (DS) and RMSD of the selected peptides with the HLA-B*4402 and HLA-A*0201 alleles.

| Peptide | Allele | Docking Score (Kcal/mol) | RMSD (Å) |
|---|---|---|---|
| EEAGRAFSF (3L3D) | HLA-B*4402 | –108.89 | 0.416 |
| GILEFVFTL (5HHP) | HLA-A*0201 | –40.53 | 0.900 |
| KQNDRCTLV | HLA-A*0201 | –60.21 | 0.825 |
| FLFWFSFGY | HLA-A*0201 | –56.85 | 1.140 |
| YLLIVVGTL | HLA-A*0201 | –37.35 | 0.570 |
| LLTVSLSPV | HLA-A*0201 | –53.81 | 0.767 |
| FVLGSILFI | HLA-A*0201 | –55.69 | 0.457 |
| TEAIVCVEL | HLA-B*4402 | –37.83 | 0.819 |

**Table S4.** Molecular docking calculations and interactions details between selected CTL epitopes and respective HLA class I allele.

| Peptide | Receptor Atoms | Receptor Residues | Receptor residues No | Peptide Atoms | Peptide Resides | Peptide residue No | Distance (Å) |
|---|---|---|---|---|---|---|---|
| **3L3D** | OH | TYR | 7 | N | GLU | 1 | 3.1 |
| | OH | TYR | 9 | OE1 | GLU | 2 | 2.55 |
| | NZ | LYS | 45 | OE2 | GLU | 2 | 2.73 |
| | NH2 | ARG | 62 | OE1 | GLU | 1 | 3.02 |
| | OE2 | GLU | 63 | N | GLU | 2 | 2.91 |
| | OD1 | ASN | 70 | N | ALA | 6 | 3 |
| | OD1 | ASN | 77 | N | PHE | 9 | 2.74 |
| | OH | TYR | 84 | O | PHE | 9 | 2.66 |
| | OH | TYR | 99 | OE1 | GLU | 2 | 2.51 |
| | OH | TYR | 99 | N | ALA | 3 | 3.05 |
| | OG1 | THR | 143 | O | PHE | 9 | 2.67 |
| | NZ | LYS | 146 | OXT | PHE | 9 | 2.83 |
| | NE1 | TRP | 147 | O | SER | 8 | 2.83 |
| | OH | TYR | 159 | O | GLU | 1 | 2.6 |
| | OG | SER | 167 | N | GLU | 1 | 3.03 |
| | NH2 | ARG | 170 | OE2 | GLU | 1 | 2.79 |
| | OH | TYR | 171 | N | GLU | 1 | 2.7 |
| **Receptor Non-Bonded Contacts:** MET5, TYR7, TYR9, THR24, LYS45, TYR59, ARG62, GLU63, ILE66, SER67, ASN70, THR73, TYR74, GLU76, ASN77, THR80, TYR84, ILE95, ARG97, TYR99, ASP116, TYR123, THR143, LYS146, TRP147, VAL152, GLN155, ASP156, TYR159, LEU163, SER167, ARG170, and TYR171. | | | | **Peptide Non-Bonded Contacts:** GLU1, GLU2, GLY4, ALA3, ALA6, PHE7, SER8, and PHE9. | | | |
| **5HHP** | OH | TYR | 7 | N | GLY | 1 | 2.83 |
| | OE1 | GLU | 63 | N | ILE | 2 | 3.09 |
| | NH1 | ARG | 65 | OE2 | GLU | 4 | 2.53 |
| | NZ | LYS | 66 | O | ILE | 2 | 2.87 |
| | OD1 | ASP | 77 | N | LEU | 9 | 2.93 |
| | OH | TYR | 99 | N | LEU | 3 | 2.98 |
| | OG1 | THR | 143 | O | LEU | 9 | 2.69 |
| | NZ | LYS | 146 | O | THR | 8 | 3.07 |
| | NZ | LYS | 146 | OG1 | THR | 8 | 3.15 |
| | NE1 | TRP | 147 | O | THR | 8 | 2.99 |
| | OH | TYR | 159 | O | GLY | 1 | 2.62 |
| | OH | TYR | 171 | N | GLY | 1 | 2.71 |
| **Receptor Non-Bonded Contacts:** TYR7, GLU63, ARG65, LYS66, VAL67, HIS70, THR73, ASP77, THR80, LEU81, TYR84, ARG97, TYR99, HIS114, TYR116, THR143, LYS146, TRP147, VAL152, LEU156, TYR159, TRP167, and TYR171. | | | | **Peptide Non-Bonded Contacts:** ILE 2, GLY1, GLU4, LEU3, VAL6, PHE7, LEU9, and THR8 | | | |
| **KQNDRCTLV** | OE2 | GLU | 63 | NE2 | GLN | 2 | 2.92 |
| | NZ | LYS | 66 | O | ASP | 4 | 2.93 |
| | NE2 | HIS | 70 | OD2 | ASP | 4 | 3.07 |
| | NH2 | ARG | 97 | O | VAL | 9 | 2.81 |
| | OH | TYR | 159 | O | ASN | 3 | 2.84 |
| | NE1 | TRP | 167 | O | LYS | 1 | 2.67 |
| **Receptor Non-Bonded Contacts:** TYR59, GLU63, LYS66, HIS70, THR73, HIS74, ASP77, ARG97, HIS114, TYR116, TYR123, THR143, TRP147, LEU156, TYR159, TRP167, and ARG170. | | | | **Peptide Non-Bonded Contacts:** LYS1, GLN2, ASP4, ASN3, LEU8, VAL9, THR7, CYS6, and ARG5. | | | |
| **FLFWFSFGY** | OG1 | THR | 73 | OH | TYR | 9 | 2.79 |
| | OG1 | THR | 73 | OH | TYR | 9 | 2.79 |
| | OD1 | ASP | 77 | N | TYR | 9 | 2.86 |
| | NE1 | TRP | 147 | OG | SER | 6 | 2.95 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Receptor Non-Bonded Contacts:** MET5, TYR7, PHE33, MET45, TYR59, GLU63, LYS66, VAL67, THR73, HIS74, ASP77, ARG97, TYR99, GLY100, TYR116, TYR123, ILE124, THR143, TRP147, VAL152, GLN155, LEU156, TYR159, CYS164, and TRP167. | | | | **Peptide Non-Bonded Contacts:** LEU2 PHE1, PHE3, PHE7, TYR9, GLY8, TRP4, SER6, and PHE5. | | | |
| **YLLIVVGTL** | OG1 | THR | 73 | OH | TYR | 9 | 2.79 |
| | OG1 | THR | 73 | OH | TYR | 9 | 2.79 |
| | OD1 | ASP | 77 | N | TYR | 9 | 2.86 |
| | NE1 | TRP | 147 | OG | SER | 6 | 2.95 |
| **Receptor Non-Bonded Contacts:** MET5, TYR7, PHE33, MET45, TYR59, GLU63, LYS66, VAL67, THR73, HIS74, ASP77, ARG97, TYR99, GLY100, TYR116, TYR123, ILE124, THR143, TRP147, VAL152, GLN155, LEU156, TYR159, CYS164, and TRP167. | | | | **Peptide Non-Bonded Contacts:** LEU2, PHE1, PHE3, PHE7, TYR9, GLY8, TRP4, SER6, and PHE5. | | | |
| **LLTVSLSPV** | OE2 | GLU | 63 | N | LEU | 1 | 2.76 |
| | NE2 | HIS | 70 | O | VAL | 4 | 3.33 |
| | OG1 | THR | 80 | O | PRO | 8 | 2.88 |
| | OG1 | THR | 143 | OXT | VAL | 9 | 2.83 |
| | NE1 | TRP | 147 | OG | SER | 7 | 2.75 |
| **Receptor Non-Bonded Contacts:** MET5, ARG6, TYR7, GLY26, PHE33, VAL34, TYR59, GLU63, LYS66, HIS70, THR73, VAL76, ASP77, THR80, TYR84, ARG97, TYR99, GLY100, HIS114, TYR123, THR143, LYS146, TRP147, VAL152, GLN155, LEU156, TYR159, CYS164, and TYR171. | | | | **Peptide Non-Bonded Contacts:** LEU2, LEU1, VAL4, THR3, LEU6, PRO8, VAL9 SER7, and SER5. | | | |
| **FVLGSILFI** | NZ | LYS | 66 | O | GLY | 4 | 2.85 |
| | NE2 | HIS | 70 | O | LEU | 3 | 3.09 |
| | NE1 | TRP | 147 | O | LEU | 7 | 2.8 |
| **Receptor Non-Bonded Contacts:** MET5, TYR7, LYS66, HIS70, THR73, HIS74, ASP77, THR80, TYR84, ARG97, TYR99, HIS114, THR143, LYS146, TRP147, VAL152, LEU156, TYR159, THR163, CYS164, and TRP167. | | | | **Peptide Non-Bonded Contacts:** PHE1, LEU3, GLY4, PHE8, ILE9, VAL2, ILE6, and LEU7. | | | |
| **TEAIVCVEL** | OE1 | GLU | 63 | N | THR | 1 | 2.75 |
| | OE1 | GLU | 63 | OG1 | THR | 1 | 2.74 |
| | NE1 | TRP | 147 | O | GLU | 8 | 2.85 |
| | OG | SER | 167 | O | GLU | 2 | 2.98 |
| | OH | TYR | 171 | N | GLU | 2 | 2.69 |
| | OH | TYR | 171 | O | GLU | 2 | 2.72 |
| **Receptor Non-Bonded Contacts:** TYR7, VAL34, ILE52, GLU55, TYR59, TRP60, GLU63, ILE66, ASN70, THR73, TYR74, ASN77, THR80, TYR99, THR143, LYS146, TRP147, VAL152, TYR159, LEU163, CYS164, SER167, and TYR171. | | | | **Peptide Non-Bonded Contacts:** ALA3, THR1, GLU2, VAL5, GLU8, LEU9, CYS6 VAL7, and ILE4. | | | |

**Table S5.** Molecular docking between selected CTL peptides and mouse MHC-I molecules

| **PDB ID: 1YN6 (classical) H2-Db** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Peptide** | **Docking Score** | **RMSD** | **Bond** | **Protein Residues** | **Peptide Residues** | **Energy (kcal/mol)** | **Distance (Å)** | **Bond-Frequency** |
| KQNDRCTLV | -40.18 | 0.92 | IH | Glu9 | Arg5 | -15.18 | 3.06 | 4 |
| | | | H | Lys66 | Asn3 | -4.20 | 2.82 | 1 |
| | | | H | Gln70 | Arg5 | -1.00 | 3.04 | 1 |
| | | | H | Asn80 | Val9 | -3.90 | 2.94 | 1 |
| | | | IH | Lys146 | Val9 | -32.39 | 2.76 | 4 |
| | | | H | Trp147 | Val9 | -6.50 | 2.92 | 1 |
| | | | H | His155 | Cys6 | -3.50 | 2.77 | 1 |
| | | | H | Tyr156 | Leu8 | -1.10 | 3.08 | 1 |
| | | | H | Gly162 | Lys1 | -5.30 | 2.73 | 1 |
| | | | H | Glu163 | Asn3 | -2.70 | 2.86 | 1 |
| | | | IH | Glu166 | Lys1 | -48.82 | 2.76 | 6 |
| | | | H | Glu166 | Asn3 | -8.00 | 2.95 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| FLFWFSFGY | -40.37 | 1.86 | H | Gln65 | Phe1 | -12.50 | 3.01 | 2 |
| | | | H | Asn80 | Tyr9 | -4.90 | 2.74 | 1 |
| | | | H | Tyr84 | Tyr9 | -3.40 | 3.01 | 2 |
| | | | IH | Lys146 | Tyr9 | -11.76 | 3.12 | 3 |
| | | | H | Trp147 | Tyr9 | -7.20 | 2.82 | 1 |
| | | | H | Ser150 | Ser6 | -1.50 | 2.84 | 1 |
| | | | H | His155 | Phe3 | -0.80 | 2.88 | 1 |
| YLLIVVGTL | -51.49 | 0.68 | H | Gln70 | Val6 | -3.10 | 2.89 | 2 |
| | | | H | Trp73 | Thr8 | -0.70 | 2.94 | 1 |
| | | | H | Gln97 | Val6 | -3.60 | 2.93 | 1 |
| | | | IH | Lys146 | Leu9 | -37.37 | 2.68 | 4 |
| | | | H | Trp147 | Thr8 | -0.70 | 2.93 | 1 |
| | | | H | His155 | Val5 | -6.50 | 2.80 | 1 |
| | | | IH | Glu163 | Tyr1 | -16.56 | 2.74 | 2 |
| | | | IH | Glu166 | Tyr1 | -32.24 | 2.76 | 4 |
| LLTVSLSPV | -36.38 | 1.75 | H | Gln65 | Thr3 | -2.60 | 2.92 | 2 |
| | | | H | Trp73 | Ser7 | -0.80 | 2.80 | 1 |
| | | | H | Asn80 | Val9 | -2.60 | 2.94 | 1 |
| | | | H | Tyr84 | Val9 | -4.30 | 2.56 | 1 |
| | | | IH | Lys146 | Val9 | -21.52 | 2.84 | 3 |
| | | | H | Trp147 | Pro8 | -0.90 | 2.79 | 1 |
| | | | H | His155 | Leu6 | -5.80 | 2.86 | 1 |
| FVLGSILFI | -35.10 | 1.92 | H | Gly69 | Leu3 | -0.60 | 3.41 | 1 |
| | | | H | Asn80 | Ile9 | -3.80 | 2.79 | 1 |
| | | | H | Tyr84 | Ile9 | -4.20 | 2.67 | 1 |
| | | | IH | Lys146 | Ile9 | -21.00 | 2.99 | 3 |
| | | | H | His155 | Phe1 | -6.70 | 3.09 | 1 |
| | | | H | Ala158 | Phe1 | -3.60 | 2.78 | 1 |
| TEAIVCVEL | -39.72 | 2.56 | H | Gln65 | Thr1 | -2.50 | 2.61 | 1 |
| | | | H | Trp73 | Val7 | -1.90 | 2.89 | 1 |
| | | | H | Ser77 | Leu9 | -1.10 | 3.28 | 1 |
| | | | H | Asn80 | Glu8 | -5.90 | 2.78 | 1 |
| | | | H | Asn80 | Leu9 | -6.80 | 2.77 | 1 |
| | | | H | Tyr84 | Leu9 | -5.20 | 2.65 | 1 |
| | | | H | Thr143 | Leu9 | -2.50 | 2.60 | 1 |
| | | | IH | Lys146 | Glu8 | -26.83 | 2.86 | 4 |
| | | | IH | Lys146 | Leu9 | -11.47 | 3.15 | 3 |
| | | | H | Trp147 | Glu8 | -1.90 | 2.83 | 1 |
| | | | H | His155 | Val5 | -4.90 | 2.83 | 1 |
| | | | H | Tyr156 | Val7 | -0.60 | 2.85 | 1 |
| **PDB ID: 6JQ3 (classical) H2-Kb** | | | | | | | | |
| TEAIVCVEL | -41.53 | 1.48 | H | Glu24 | Gln2 | -3.80 | 2.90 | 1 |
| | | | IH | Arg62 | Asp4 | -15.23 | 3.07 | 4 |
| | | | H | Lys66 | Gln2 | -8.10 | 2.78 | 1 |
| | | | H | Gly69 | Lys1 | -8.40 | 2.83 | 1 |
| | | | H | Gln72 | Lys1 | -6.50 | 2.70 | 1 |
| | | | IH | Glu152 | Lys1 | -28.28 | 2.71 | 4 |
| | | | H | Thr163 | Asp4 | -1.70 | 2.69 | 1 |
| | | | IH | Glu166 | Arg5 | -35.72 | 2.82 | 6 |
| | | | H | Glu166 | Thr7 | -3.60 | 2.66 | 1 |
| | | | H | Trp167 | Asp4 | -5.30 | 2.80 | 1 |
| | | | H | Arg169 | Leu8 | -6.90 | 2.82 | 1 |
| | | | IH | Lys173 | Val9 | -41.75 | 2.67 | 4 |
| TEAIVCVEL | -44.40 | 1.78 | H | Tyr7 | Tyr9 | -4.80 | 2.61 | 1 |
| | | | H | Arg62 | Phe7 | -4.40 | 2.77 | 1 |
| | | | IH | Lys66 | Tyr9 | -24.63 | 2.70 | 2 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | H | Asn70 | Tyr9 | -3.20 | 2.71 | 1 |
| | | | H | Lys146 | Phe1 | -8.20 | 2.86 | 1 |
| | | | H | Gln149 | Phe1 | -9.80 | 2.80 | 2 |
| | | | H | Arg155 | Phe3 | -0.80 | 3.05 | 1 |
| | | | H | Tyr159 | Tyr9 | -1.70 | 2.61 | 1 |
| TEAIVCVEL | -39.64 | 1.15 | H | Arg62 | Val6 | -4.20 | 2.79 | 1 |
| | | | H | Lys66 | Ile4 | -11.40 | 2.79 | 1 |
| | | | H | Ser73 | Tyr1 | -2.40 | 2.81 | 1 |
| | | | IH | Asp77 | Tyr1 | -26.87 | 2.77 | 3 |
| | | | H | Tyr116 | Tyr1 | -3.10 | 2.66 | 1 |
| | | | H | Arg155 | Leu2 | -2.40 | 2.85 | 1 |
| | | | H | Arg155 | Leu3 | -2.70 | 2.76 | 1 |
| | | | H | Glu166 | Thr8 | -1.40 | 2.69 | 1 |
| | | | H | Trp167 | Gly7 | -2.50 | 2.91 | 1 |
| | | | IH | Arg169 | Leu9 | -28.73 | 3.04 | 6 |
| | | | H | Arg170 | Leu9 | -0.70 | 3.40 | 1 |
| TEAIVCVEL | -49.25 | 0.92 | H | Glu24 | Thr3 | -1.30 | 2.71 | 1 |
| | | | H | Arg62 | Leu6 | -1.30 | 2.84 | 1 |
| | | | H | Arg62 | Ser7 | -8.30 | 2.84 | 2 |
| | | | H | Lys66 | Val4 | -12.20 | 2.76 | 1 |
| | | | IH | Asp77 | Leu1 | -26.31 | 2.84 | 4 |
| | | | H | Ser99 | Thr3 | -1.60 | 2.93 | 1 |
| | | | H | Tyr116 | Leu1 | -3.30 | 2.71 | 1 |
| | | | H | Tyr159 | Ser5 | -2.20 | 2.76 | 1 |
| | | | H | Trp167 | Leu6 | -1.70 | 2.73 | 1 |
| TEAIVCVEL | -36.77 | 0.85 | H | Tyr7 | Ile9 | -4.70 | 2.61 | 1 |
| | | | I | Arg62 | Ile9 | -2.58 | 3.34 | 1 |
| | | | IH | Lys66 | Ile9 | -15.16 | 2.81 | 2 |
| | | | H | Ser73 | Phe1 | -4.50 | 2.95 | 1 |
| | | | IH | Asp77 | Phe1 | -18.98 | 2.81 | 3 |
| | | | H | Arg155 | Leu3 | -9.50 | 2.88 | 2 |
| | | | H | Arg155 | Ile6 | -5.30 | 2.78 | 1 |
| | | | H | Tyr159 | Ile9 | -1.60 | 2.63 | 1 |
| TEAIVCVEL | -34.10 | 1.10 | IH | Arg62 | Leu9 | -32.02 | 2.97 | 7 |
| | | | IH | Lys66 | Leu9 | -14.94 | 2.69 | 2 |
| | | | H | Asn70 | Val7 | -3.90 | 2.87 | 1 |
| | | | H | Tyr116 | Cys6 | -2.30 | 3.30 | 1 |
| | | | H | His145 | Thr1 | -1.70 | 2.89 | 1 |
| | | | H | Lys146 | Glu2 | -0.60 | 3.26 | 1 |
| | | | H | Lys146 | Ala3 | -3.50 | 2.86 | 1 |
| | | | H | Gln149 | Thr1 | -16.20 | 2.83 | 2 |
| | | | H | Glu152 | Cys6 | -8.70 | 3.03 | 2 |
| | | | IH | Arg155 | Glu8 | -35.32 | 3.00 | 6 |
| | | | H | Trp167 | Leu9 | -2.70 | 2.80 | 1 |
| **PDB ID: 7LFK (non-classical) H2-M3** | | | | | | | | |
| TEAIVCVEL | -51.22 | 1.34 | IH | Glu62 | Lys1 | -29.07 | 2.77 | 4 |
| | | | H | Glu62 | Gln2 | -7.40 | 2.79 | 1 |
| | | | H | Asn77 | Val9 | -5.80 | 2.95 | 2 |
| | | | H | Thr143 | Val9 | -2.30 | 2.60 | 1 |
| | | | H | Ala150 | Arg5 | -4.60 | 2.79 | 1 |
| | | | H | Ala158 | Asn3 | -4.70 | 3.05 | 2 |
| | | | H | Gly162 | Lys1 | -10.80 | 2.79 | 1 |
| | | | IH | Glu166 | Lys1 | -16.49 | 2.62 | 2 |
| TEAIVCVEL | -52.97 | 1.61 | H | Ser73 | Ser6 | -1.50 | 2.85 | 1 |
| | | | H | Asn77 | Phe7 | -1.90 | 2.85 | 1 |
| | | | H | Tyr155 | Trp4 | -2.30 | 2.96 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | H | Ala158 | Phe1 | -10.20 | 2.91 | 1 |
| | | | IH | Glu161 | Phe1 | -28.70 | 2.81 | 3 |
| TEAIVCVEL | -48.01 | 1.01 | H | Ala76 | Leu9 | -0.50 | 3.54 | 1 |
| | | | H | Asn77 | Gly7 | -0.50 | 2.99 | 1 |
| | | | IH | Arg79 | Leu9 | -27.06 | 3.09 | 6 |
| | | | H | Thr80 | Thr8 | -1.50 | 2.95 | 1 |
| | | | H | Arg146 | Val6 | -7.10 | 2.76 | 1 |
| | | | H | Arg146 | Thr8 | -5.80 | 2.94 | 2 |
| | | | H | Ala158 | Tyr1 | -0.50 | 2.86 | 1 |
| | | | A | Glu163 | Tyr1 | -0.50 | 3.43 | 1 |
| | | | H | Glu166 | Tyr1 | -5.20 | 2.62 | 1 |
| TEAIVCVEL | -36.17 | 1.02 | H | Asn77 | Ser5 | -2.00 | 2.72 | 1 |
| | | | H | Asn77 | Ser7 | -2.30 | 2.86 | 1 |
| | | | H | Thr80 | Val9 | -2.10 | 2.71 | 1 |
| | | | H | Arg146 | Ser7 | -4.10 | 2.74 | 1 |
| | | | IH | Arg146 | Val9 | -24.39 | 2.97 | 5 |
| | | | H | Ala158 | Leu1 | -3.50 | 2.68 | 1 |
| | | | IH | Glu163 | Leu1 | -18.17 | 2.58 | 2 |
| TEAIVCVEL | -43.89 | 0.70 | H | Lys66 | Leu3 | -9.00 | 2.80 | 1 |
| | | | H | Asn77 | Ile9 | -4.80 | 2.75 | 1 |
| | | | H | Tyr114 | Ile9 | -4.20 | 2.61 | 1 |
| | | | H | Gly162 | Phe1 | -2.90 | 3.00 | 1 |
| | | | IH | Glu166 | Phe1 | -24.68 | 2.65 | 2 |
| TEAIVCVEL | -31.13 | 1.73 | H | Asn77 | Leu9 | -6.60 | 2.83 | 1 |
| | | | H | Thr80 | Leu9 | -0.60 | 2.55 | 1 |
| | | | H | Thr143 | Glu8 | -1.00 | 2.65 | 1 |
| | | | H | Arg146 | Val7 | -4.50 | 2.81 | 1 |
| | | | IH | Arg146 | Leu9 | -24.79 | 2.75 | 4 |
| | | | H | Thr152 | Cys6 | -1.70 | 3.20 | 1 |
| | | | H | Glu161 | Thr1 | -2.20 | 2.62 | 1 |

**Table S6.** Atom-atom interactions across TLR2– designed vaccine construct interface obtained using PDBsum server

| S. No | TLR2 Atoms | TLR2 Residues | TLR2 residues No | Vaccine Atoms | Vaccine Resides | Vaccine residue No | Distance (Å)-bond type (H=H-bond) |
|---|---|---|---|---|---|---|---|
| 1 | OE1 | GLN | 79 | N | ARG | 246 | 3.05-H |
| 2 | OG1 | THR | 127 | OG1 | THR | 167 | 3.03-H |
| 3 | NZ | LYS | 150 | OE2 | GLU | 58 | 2.73-H |
| 4 | NE2 | GLN | 152 | OE1 | GLU | 131 | 3.11-H |
| 5 | OD1 | ASN | 248 | OG1 | THR | 126 | 3.14-H |
| 6 | ND2 | ASN | 248 | O | ALA | 125 | 2.88-H |
| 7 | NZ | LYS | 252 | O | GLU | 68 | 2.81-H |
| 8 | NZ | LYS | 308 | O | ALA | 70 | 2.94-H |
| 9 | O | LYS | 551 | NZ | LYS | 318 | 2.78-H |
| 10 | OG | SER | 553 | N | VAL | 321 | 3.06-H |
| 11 | OG | SER | 553 | O | VAL | 321 | 3.2-H |
| 12 | O | GLN | 554 | OG1 | THR | 294 | 2.95-H |
| 13 | OE1 | GLN | 554 | N | TRP | 319 | 3.02-H |
| 14 | NE2 | GLN | 554 | O | CYS | 296 | 2.84-H |
| 15 | NE2 | GLN | 557 | O | VAL | 292 | 2.81-H |
| 16 | NH1 | ARG | 570 | O | TYR | 323 | 2.97-H |
| 17 | NH2 | ARG | 570 | O | TYR | 323 | 2.82-H |
| 18 | NH2 | ARG | 570 | O | ILE | 324 | 3.18-H |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 19 | NZ | LYS | 150 | OE1 | GLU | 58 | 2.73-salt bridge |
| 20 | NZ | LYS | 252 | OE2 | GLU | 68 | 3.97-salt bridge |
| **TLR2 Non-Bonded Contacts:** ARG32, ASN33, ILE35, SER56, GLN79, ALA80, GLU103, HIS104, SER125, THR127, PHE128, LYS150, GLN152, ASN248, LEU250, LYS252, LYS308, LYS527, LYS551, SER553, GLN554, GLN557, GLY558, SER559, and ARG570 | | | | **Vaccine Non-Bonded Contacts:** HIS220, CYS245, MET247, ARG246, SER169, THR167, SER248, GLY249, PRO250, GLU58, GLU131, THR126, ALA125, GLU68, ALA70, GLY293, LYS318, VAL321, THR294, TRP319, CYS296, THR297, VAL292, TYR323, and ILE324 | | | |

**Table S7.** Atom-atom interactions across TLR3– designed vaccine construct interface obtained using PDBsum server

| S. No | TLR3 Atoms | TLR3 Residues | TLR3 residues No | Vaccine Atoms | Vaccine Resides | Vaccine residue No | Distance (Å) |
|---|---|---|---|---|---|---|---|
| 1 | NZ | LYS | 27 | OE2 | GLU | 33 | 2.7-H |
| 2 | NE2 | HIS | 32 | O | ALA | 38 | 2.89-H |
| 3 | OG1 | THR | 78 | O | GLY | 310 | 2.72-H |
| 4 | OD2 | ASP | 81 | OG | SER | 152 | 2.65-H |
| 5 | NZ | LYS | 102 | O | PRO | 311 | 2.75-H |
| 6 | NZ | LYS | 102 | O | GLY | 312 | 2.75-H |
| 7 | ND2 | ASN | 105 | O | GLY | 151 | 3.05-H |
| 8 | OE1 | GLN | 107 | NZ | LYS | 153 | 2.92-H |
| 9 | NE2 | GLN | 107 | O | GLY | 151 | 2.77-H |
| 10 | OE1 | GLU | 175 | NZ | LYS | 145 | 2.66-H |
| 11 | NZ | LYS | 200 | O | GLY | 199 | 2.74-H |
| 12 | OD1 | ASN | 230 | NZ | LYS | 94 | 2.87-H |
| 13 | NH1 | ARG | 251 | OD2 | ASP | 202 | 2.77-H |
| 14 | NH2 | ARG | 251 | OD2 | ASP | 202 | 2.73-H |
| 15 | ND2 | ASN | 252 | OG | SER | 201 | 2.9-H |
| 16 | OH | TYR | 283 | O | ASP | 98 | 2.78-H |
| 17 | OH | TYR | 307 | N | ALA | 70 | 2.97-H |
| 18 | OH | TYR | 326 | OE1 | GLN | 209 | 2.8-H |
| 19 | NZ | LYS | 330 | O | GLU | 68 | 2.69-H |
| 20 | NZ | LYS | 330 | OE1 | GLU | 68 | 2.73-H |
| 21 | NH1 | ARG | 331 | O | ALA | 70 | 2.75-H |
| 22 | NH1 | ARG | 331 | O | GLY | 124 | 2.75-H |
| 23 | NH2 | ARG | 331 | O | GLY | 124 | 2.99-H |
| 24 | NE2 | HIS | 359 | NH1 | ARG | 212 | 3.09-H |
| 25 | NZ | LYS | 416 | OE1 | GLU | 68 | 2.86-H |
| 26 | NZ | LYS | 416 | OE2 | GLU | 68 | 2.69-H |
| 27 | OE1 | GLU | 434 | OG1 | THR | 213 | 2.76-H |
| 28 | OE2 | GLU | 434 | N | GLY | 251 | 2.81-H |
| 29 | OE1 | GLU | 533 | OG | SER | 169 | 2.58-H |
| 30 | ND1 | HIS | 565 | OG | SER | 169 | 3-H |
| 31 | NZ | LYS | 589 | O | SER | 169 | 2.89-H |
| 32 | NZ | LYS | 589 | O | LEU | 170 | 2.83-H |
| 33 | NZ | LYS | 589 | OG | SER | 171 | 2.88-H |
| 34 | O | SER | 611 | OG | SER | 171 | 2.71-H |
| 35 | NZ | LYS | 613 | O | SER | 171 | 2.96-H |
| 36 | OG1 | THR | 638 | O | PHE | 13 | 2.84-H |
| 37 | NZ | LYS | 27 | OE1 | GLU | 33 | 2.7--salt bridge |
| 38 | NZ | LYS | 102 | OD1 | ASP | 313 | 2.76-salt bridge |
| 39 | NE2 | HIS | 156 | OE2 | GLU | 92 | 2.88-salt bridge |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 40 | OE1 | GLU | 175 | NZ | LYS | 145 | 2.66-salt bridge |
| 41 | NH2 | ARG | 251 | OD1 | ASP | 202 | 2.73-salt bridge |
| 42 | NZ | LYS | 330 | OE2 | GLU | 68 | 2.73-salt bridge |
| 43 | NH1 | ARG | 331 | OD1 | ASP | 72 | 3.84-salt bridge |
| 44 | NZ | LYS | 416 | OE1 | GLU | 68 | 2.69-salt bridge |
| 45 | NE2 | HIS | 665 | OE1 | GLU | 15 | 2.7-salt bridge |
| **TLR3 Non-Bonded Contacts:** LYS27, VAL30, SER31, HIS32, VAL34, THR54, VAL55, ASN57, THR59, THR78, SER79, ASP81, LYS102, ASN105, GLN107, GLU127, HIS156, GLN174, GLU175, ASN180, LYS200, ASN230, ARG251, ASN252, TYR283, TYR302, TYR307, TYR326, LYS330, ARG331, GLU358, HIS359, TYR383, ILE411, LYS416, GLU434, PHE459, GLN483, GLU533, HIS565, ILE566, GLU587, LYS589, ILE590, SER611, LYS613, LEU637, THR638, HIS665, THR666, and ASN667 | | | | **Vaccine Non-Bonded Contacts:** GLU33, VAL34, THR35, ALA38, PRO309, VAL42, GLY310, PRO311, SER152, ALA148, GLY151, GLY312, ASP313, LYS153, ALA147, LYS91, GLU92, GLY199, GLY198, LYS145, LEU197, LYS94, ASP95, ASP202, SER201, LYS74, ASP98, ALA70, ALA100, SER206, ALA69, GLN209, GLU68, ASP72, GLY124, GLY71, LEU210, ARG212, THR213, GLY251, GLY249, PRO250, SER248, MET247, SER169, GLU58, SER171, LEU170, LYS14, PHE13, ASP11, and GLU15 | | | |

**Table S8.** Atom-atom interactions across TLR4–designed vaccine construct interface obtained using PDBsum server

| S. No | TLR4 Atoms | TLR4 Residues | TLR4 residues No | Vaccine Atoms | Vaccine Resides | Vaccine residue No | Distance (Å) |
|---|---|---|---|---|---|---|---|
| 1 | O | VAL | 32 | OG1 | THR | 126 | 2.9-H |
| 2 | OE1 | GLU | 42 | NE | ARG | 212 | 2.77-H |
| 3 | OD2 | ASP | 84 | NE2 | GLN | 209 | 2.83-H |
| 4 | NE | ARG | 87 | O | LEU | 210 | 2.73-H |
| 5 | O | HIS | 159 | SG | CYS | 289 | 3.29-H |
| 6 | NH1 | ARG | 234 | O | GLY | 199 | 2.79-H |
| 7 | NH1 | ARG | 234 | OD2 | ASP | 202 | 2.72-H |
| 8 | NH2 | ARG | 234 | O | GLY | 199 | 2.96-H |
| 9 | NH2 | ARG | 264 | O | VAL | 193 | 2.89-H |
| 10 | O | ASN | 265 | N | GLY | 295 | 3.01-H |
| 11 | ND2 | ASN | 265 | OD2 | ASP | 203 | 2.84-H |
| 12 | OE1 | GLU | 266 | N | THR | 294 | 2.78-H |
| 13 | OE1 | GLU | 270 | NZ | LYS | 318 | 2.65-H |
| 14 | NH1 | ARG | 289 | O | GLY | 198 | 2.79-H |
| 15 | OH | TYR | 296 | O | TYR | 314 | 2.69-H |
| 16 | OG | SER | 317 | OE1 | GLU | 196 | 2.5-H |
| 17 | OG1 | THR | 319 | O | ASP | 313 | 2.91-H |
| 18 | NE | ARG | 322 | OD2 | ASP | 313 | 2.8-H |
| 19 | NH2 | ARG | 322 | OD2 | ASP | 313 | 2.74-H |
| 20 | NZ | LYS | 341 | O | PRO | 311 | 2.78-H |
| 21 | NZ | LYS | 341 | O | GLY | 312 | 2.85-H |
| 22 | OD2 | ASP | 502 | N | LEU | 90 | 3-H |
| 23 | NE2 | GLN | 505 | O | SER | 86 | 2.8-H |
| 24 | ND2 | ASN | 526 | O | GLY | 87 | 2.84-H |
| 25 | O | LEU | 549 | NZ | LYS | 79 | 2.91-H |
| 26 | ND2 | ASN | 575 | OE1 | GLU | 83 | 2.69-H |
| 27 | NE2 | GLN | 578 | OE2 | GLU | 115 | 2.81-H |
| 28 | OE2 | GLU | 603 | NZ | LYS | 119 | 2.69-H |
| 29 | OE2 | GLU | 605 | NZ | LYS | 119 | 2.7-H |
| 30 | NE | ARG | 606 | OE2 | GLU | 115 | 2.81-H |
| 31 | NH1 | ARG | 606 | O | GLU | 83 | 3.11-H |
| 32 | NH2 | ARG | 606 | O | GLU | 83 | 2.84-H |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 33 | OE2 | GLU | 42 | NH1 | ARG | 212 | 2.75-salt bridge |
| 34 | NH1 | ARG | 234 | OD1 | ASP | 202 | 2.72-salt bridge |
| 35 | OE1 | GLU | 270 | NZ | LYS | 318 | 2.65-salt bridge |
| 36 | NH2 | ARG | 322 | OD2 | ASP | 313 | 2.74-salt bridge |
| 37 | OE2 | GLU | 603 | NZ | LYS | 119 | 2.69-salt bridge |
| 38 | OE1 | GLU | 605 | NZ | LYS | 119 | 2.7-salt bridge |
| 39 | NE | ARG | 606 | OE1 | GLU | 115 | 2.81-salt bridge |
| **TLR4 Non-Bonded Contacts:** VAL32, MET41, GLU42, PHE63, ASP84, SER86, ARG87, THR110, GLU135, HIS159, LEU161, LYS186, LEU212, ARG234, PHE263, ARG264, ASN265, GLU266, GLY267, GLU270, ARG289, TYR292, ASP294, TYR296, VAL316, SER317, THR319, GLU321, ARG322, ASN339, LYS341, SER360, LYS362, ASN383, PHE408, LYS477, PHE500, ASP502, SER504, GLN505, ASN526, SER528, HIS529, VAL548, LEU549, ASP550, SER552, LEU553, PHE573, ASN575, THR577, GLN578, VAL602, GLU603, GLU605, and ARG606 | | | | **Vaccine Non-Bonded Contacts:** THR126, ARG212, THR213, GLN209, LEU210, PHE257, CYS289, ARG207, ASP202, GLY199, ASP203, TYR263, LEU306, VAL193, GLY295, TRP142, GLY293, THR294, VAL321, PHE316, LYS318, GLY198, TYR314, ASP313, GLU196, GLY312, LYS145, ALA147, VAL195, PRO311, ALA148, GLY151, SER152, LYS153, LEU90, GLY89, GLY87, SER86, LYS79, GLU83, ARG82, ILE84, GLU115, VAL80, and LYS119 | | | |

**Table S9.** Fraction of hydrogen bonds at the designed vaccine construct–TLRs interface during the 100ns simulation timescale (with occupancy ≥ 2%)

| **Modeled Vaccine-TLR2 complex** | | | |
|---|---|---|---|
| **TLR2 resdiue@atom: Vaccine construct residue@atom** | **No. of frames** | **Frac.** | **Avg** |
| GLU77@OE2: VAL168@N | 5970 | 54.3 | 2.88 |
| ARG544@NH2: GLU322@O | 2586 | 23.5 | 2.87 |
| LYS124@NZ: ALA57@OE1 | 2391 | 21.7 | 2.81 |
| LYS282@NZ: ALA69@O | 1273 | 11.6 | 2.82 |
| GLN531@NE2: GLY293@OG1 | 802 | 7.29 | 2.89 |
| GLN528@O: GLY293@OG1 | 756 | 6.87 | 2.86 |
| GLU77@OE2: VAL168@OG | 747 | 6.79 | 2.69 |
| LYS525@O: VAL317@NZ | 587 | 5.34 | 2.81 |
| GLN528@NE2: GLY295@O | 355 | 3.23 | 2.87 |
| SER533@OG: GLU322@O | 227 | 2.06 | 2.91 |
| **Modeled Vaccine-TLR3 complex** | | | |
| **TLR3 resdiue@atom: Vaccine construct residue@atom** | **No. of frames** | **Frac.** | **Avg** |
| ASN36@ND2: GLY151@OG | 4159 | 37.8 | 2.88 |
| LYS81@NZ: GLY310@O | 2711 | 24.6 | 2.83 |
| GLU512@OE1: VAL168@OG | 2425 | 22 | 2.71 |
| PRO230@NH2: SER201@OD1 | 2238 | 20.3 | 2.83 |
| LYS81@NZ:PRO311@O | 2132 | 19.4 | 2.83 |
| LYS81@NZ: GLY312@OD1 | 1409 | 12.8 | 2.8 |
| LYS309@NZ: LEU67@O | 1342 | 12.2 | 2.82 |
| HIS544@ND1: VAL168@OG | 1218 | 11.1 | 2.85 |
| ASP60@OD2: GLY151@OG | 1134 | 10.3 | 2.68 |
| GLU154@OE1: LEU144@NZ | 1108 | 10.1 | 2.79 |
| TYR262@OH: VAL97@O | 844 | 7.67 | 2.78 |
| HIS644@NE2: LYS14@OE1 | 783 | 7.12 | 2.79 |
| ARG310@NH1: ALA123@O | 754 | 6.85 | 2.83 |
| GLU154@OE2: LEU144@NZ | 539 | 4.9 | 2.84 |
| LYS6@NZ: PHE32@OE1 | 408 | 3.71 | 2.8 |
| GLU413@OE2:PRO250@N | 289 | 2.63 | 2.86 |
| **Modeled Vaccine-TLR4 complex** | | | |
| **TLR4 resdiue@atom: Vaccine construct residue@atom** | **No. of frames** | **Frac.** | **Avg** |
| ASN313@ND2: CYS194@O | 7296 | 66.3 | 2.85 |
| ASN549@ND2: ARG82@OE2 | 4825 | 43.9 | 2.82 |
| GLU16@OE2: LEU211@NH1(63%) | 4595 | 41.8 | 2.81 |
| SER291@OG: VAL195@OE2 | 3175 | 28.9 | 2.71 |
| ARG296@NH2: GLY312@OD2(47%) | 2800 | 25.5 | 2.82 |
| ARG296@NE: GLY312@OD2 | 2494 | 22.7 | 2.81 |
| GLU16@OE1: LEU211@NE | 2379 | 21.6 | 2.84 |
| LYS315@NZ: LEU311@O | 1984 | 18 | 2.83 |
| GLU244@OE1: VAL317@NZ | 1874 | 17 | 2.81 |
| GLU577@OE2: ALA118@NZ | 1519 | 13.8 | 2.79 |
| ARG580@NH2: ARG82@O | 1438 | 13.1 | 2.85 |
| GLU240@OE2: GLY293@N | 1192 | 10.8 | 2.86 |
| TYR270@OH:ASP313@O | 937 | 8.52 | 2.7 |
| ARG208@NH1: GLY198@O | 691 | 6.28 | 2.82 |
| LYS315@NZ: GLY310@O | 559 | 5.08 | 2.83 |
| LEU523@O: ILE78@NZ | 432 | 3.93 | 2.88 |
| ARG208@NH1: SER201@OD1 | 241 | 2.19 | 2.8 |