Original

Design of Multi-Epitope Vaccine from Nipah Virus proteome using Immunoinformatics approach

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Abstract:

Background: Nipah virus (NiV) is a zoonotic virus of the paramyxovirus family that sporadically breaks out from livestock and human. Annual review of diseases prioritized under the R&D WHO Blueprint listed the Nipah virus as one of the emerging infectious diseases requiring urgent research and development effort. Moreover, there is a major layback in the development of effective vaccines or drugs against NiV. The aim of this study is to design a non-toxic and potent multi-epitope vaccine against NiV using bioinformatics approach.

Materials & Method: In this study, T and B-cell epitopes of NiV protein were predicted and screened based on the antigenicity, toxicity, allergenicity and IFN induction features. The epitopes were linked by suitable linkers. Four different adjuvants were attached to the vaccine constructs, then secondary and the 3D structure of the vaccines was predicted. The refinement process was performed to improve the quality of the 3D model structure; the validation process performed using the Ramachandran plot. The designed vaccines binding affinity to HLA molecules, TLR 8 and TLR were evaluated by molecular docking, and select the best docking score for all vaccines against TLR8. Finally, in silico gene cloning was performed in the pET28a (+) vector**.**

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Result: The proposed vaccine was shown to be antigenic in VaxiJen server, non-allergenic in Allertop server and non-toxic in Toxinpred server. Thephysiochemical properties of the vaccine showed constructed vaccines stable and can be soluble overexpression.Regarding MHC-I, predicted epitopes for studied proteins (G, FG, M, N, P, L, V and W) ranged from 6 to 52, with an IC_{50} from 1 to 100, while in MHC-II, predicted epitopes ranged from 1 to 61 with an IC_{50} 1 to 500. Vaccine tertiary structure was predicted, refined and validated to assess the stability of the vaccine via Ramachandran plot. Moreover, solubility of the vaccine construct was greater than the average solubility provided by protein SOL and SOLpro servers. Disulfide engineering was performed to reduce the high mobile regions in the vaccine to enhance stability which was predicted for the vaccines constructed, except L proposed vaccine which was dispensed. Docking of the vaccine construct with TLR8 showed the best binding energy with all proposed vaccines, TLR8 ligand result was taken for all next studies. Immune-simulation significantly provided high levels of immunoglobulins, T-helper cells, T-cytotoxic cells and INF-γ. Upon cloning, the vaccine protein was reverse transcribed into DNA sequence and cloned into pET28a (+) vector to ensure translational potency and microbial expression. **Conclusion:** The overall results of the study proved that the multi-epitope construct is a potential candidate for an efficient protective vaccine against NiV. The immunoinformatics approaches accelerate vaccine development process to reduce the risk of in vitro pre-clinical trials.

Keywords: Nipah virus, vaccine, immune-simulation, immune-informatics, molecular dynamics.

Introduction

Nipah virus (NiV) is a bat-borne virus that was discovered 20 years ago in Malaysia, following an outbreak in South and Southeast Asia (1).NiV is a member of the genus Henipavirus of the family Paramyxoviridae (1). NiV initially spreads between animals and people; therefore it is highly infectious. The infection caused by NiV is highly fatal, as it causes neurological and respiratory disease (1). The Nipah virus bears a negative-stranded RNA genome consisting of six essential genes, glycoprotein (G), fusion glycoprotein (F), matrix (M), phosphoprotein (P), nucleocapsid (N), and long polymerase (L). The P, N and L attached to the viral RNA forming the virus ribonucleoprotein (vRNP). G and F proteins are responsible for cellular attachment of the virion and subsequent host cell entry (2). The G glycoprotein mediates attachment to host cellular surface receptors and the fusion (F) protein makes fusion of virus- cellular membranes for cell entry. The G protein of NiV binds to host ephrin B2/3 receptors and induces conformational modifications in G protein that cause the F protein refolding (3).The virus M protein mediates morphogenesis and budding (4), (5). It is vital to be aware that among the proteins in the matrix is the preservation of the molecular details of the virus (6). The NiV V protein is one of the three accent proteins encoded via way of means of the viral P gene, performs important position in pathogenesis of the virus in experimental infection in hamster (7). NiV V protein has been proven to boom the extent of a number of UBXN1 (UBX domain-containing protein 1, a negative controller of RIG-I-like receptor signaling) through restraining its proteolysis,

and therefore regulate (suppress) innate int erferon induction (7), (8). T cell epitopes are typically protein antigen-derived peptides provided via way of means of essential histocompatibility complex (MHC) molecules on antigen-presenting cells. These are diagnosed through means of T-cell receptors and consequently, called T-cell epitopes. B-cell epitopes are capable of binding to an antibody, these two cells mediate adaptive immunity, i.e. extend the memory specific to the pathogen. This invokes the immunological protection (9). In- silico, immunoinformatic approach offers a promising solution towards novel vaccine design. The method involves identification of antigenic epitopes within antigenic proteins which can be used to elicit both cellular and humoral immune responses (10). It works by the specific interaction between the isolated antigenic

epitopes with Major Histocompatibility Complex (MHC) and Human Leukocyte Antigen (HLA) molecules during antigen presentation (11). Epitope based vaccines eliminate the risk associated with attenuation and add the benefit of an in-silico validation prior to in vitro trials [10], (11). They also reduce the time and cost associated with the vaccine development. Surface glycoproteins such as G protein and F protein are considered ideal targets for the immune system due to their interaction with the host cell receptors and exposure on the viral surface. In this study the whole Nipah virus proteome was to find out the most antigenic, non-allergic, non-toxic and immune inducing epitopes to construct different vaccines that undergone deep investigation to reveal the most appropriate vaccine.

Material and Methods

Protein Sequences Retrieval

The whole proteins of NiV (G, FG, M, P, N, L, V and W) were retrieved from NCBI (National Center for Biotechnology Information(nih.gov)) and UniProt (http://www.uniprot.org/uniprot/?query=nipah+virus).

The Proteins alignment and physicochemical properties Predication

The alignment of proteins was done by using Clustal-W byBio-edit software program version (11). Then the physicochemical properties of the proteins such as the molecular weight, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) etc. were determined using Protparam tool (ExPASy - ProtParam tool)(12), (13).

T-cell and B-cell Prediction

The T and B cell epitopes were predicted by using the Immune Epitope Database (IEDB) (https://www.iedb.org/), which contains an enormous series of experimental records on T-cell epitopes and antibodies (14).

Peptide Binding to MHC Class I and MHC Class II Molecules for T-cell

The MHC class-I and MHC class-II epitopes were predicted using the IEDB prediction tool at(MHC-IBinding (http://tools.iedb.org/mhci/) andMHC-II Binding (http://tools.iedb.org/mhcii/), respectively (15).

Assessment of Antigenicity, Allergenicity, Toxic properties and Human Homology of the Multi-epitope vaccine candidate

The antigenicity, allergenicity, toxicity of the epitopes were done using VaxiJen v2.0 **Error! Hyperlink reference not valid.**(16), AllerTOPv2.0 (https://www.ddgpharmfac.net/AllerTOP/)

and Toxinpred (https://webs.iiitd.edu.in/raghava/toxinpred/design.php) (17), (18) respectively. The homology of human proteome epitopes has moreover changed in this step, in which Homo sapiens (taxid: 9606) was used for assessment the protein BLAST module (blast P) (19). (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Prediction of Interferon-γ (IFN-γ) targeted epitopes and Solubility Prediction

IFN-gamma induction by epitopes used to select best MHC II epitopes and examine via the online IFN epitope tool, (https://webs.iiitd.edu.in/raghava/ifnepitope/developer.php) (20).The solubility of the constructed vaccine upon expression in Escherichia coli was determined by using SOLpro (http://scratch.proteomics.ics.uci.edu/)(21)

Tertiary Structure Refinement and Validation and Vaccine Construction

The 3D structure of the G, FG, M, P, N, L, V and W proteins was predicted by using the SWISS-MODEL software (SWISS-MODEL Interactive Workspace(expasy.org).The generated 3D structure of the proteins was refined by GalaxyRefine module of SWISS-MODEL software (SWISS-MODEL Interactive Workspace (expasy.org)(22). The refinement and validation of the 3D were done by using Ramachandran plots, generated by PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/) server (23). The vaccine constructs have been admixed in exclusive mixtures with the assist of GGGGS, GPGPG, KK, GG, AAY, and EAAAK linkers (24), (25).

Molecular Docking of Multi-epitope vaccine candidate with Toll like Receptor and allele

Molecular docking was performed between the MHC allele HLA-A*02:01 (PDB ID: 4U6X) and (TLR8 PDB ID:3W3M) and (TLR PDB ID:6SWS) downloaded from Protein Databank (PDB) (https://www. rcsb.org), using FireDock server (http://bioinfo3d.cs.tau.ac.il/FireDock/php.php). HawkDock server examine the structures of PPIs with the aid of using integrating the ATTRACT docking algorithm, the HawkDock scoring function and the MM/GBSA free energy decomposition analysis (HawkDock Server(zju.edu.cn) (26) . Moreover, ClusPro 2.0 (ClusPro 2.0: protein-protein docking) was used for molecular docking and docking refinement.

Secondary Structure Prediction of the proteins

The secondary structures of the proteins were predicted by using two online tools, PRISPRED(http://bioinf.cs.ucl.ac.uk/psipred/) and SOPMA (https://npsa-prabi.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) (27), (28).

Vaccine Protein Disulfide Engineering

The disulfide bond was conducted by using DbD 2 v12.2 (http://cptweb.cpt.wayne.edu/DbD2/) server (29).

Screening for Conformational B-lymphocytic Epitopes

The conformational B-cell epitopes of the best-expected vaccine protein had been determined with the aid of using IEDB ElliPro tool (http://tools.iedb.org/ellipro/), used the default parameters of a minimum score of 0.5 and a maximum distance of 6 angstrom (30).

Molecular Dynamics Simulation Analysis and Immune Simulation

The molecular dynamics simulation examine was carried out through the online simulation tool, CABS-flex 2.0 (http://biocomp.chem.uw.edu.pl/CABSflex2/).(31), (32). The immune simulation was carried out by using C-ImmSim server (http://150.146.2.1/CIMMSIM/index.php) (33).

Codon Adaptation, In Silico Cloning, Prediction of the mRNA Secondary Structure, and Expression of the best Vaccine Protein with SUMO-fusion

The Java Codon Adaptation Tool and JCat server (http://www.jcat.de/) were used for codon adaptation examine (34). Then the Prokaryotic E. coli strain K12 was selected as the target organism and Rho independent transcription terminators, prokaryotic ribosome binding sites and BamHI and EcoRI cleavage sites of restriction enzymes, were avoided at the server. The vaccine protein sequences become oppositely translated to the optimized DNA sequence through the server. The optimized sequence of the final vaccine construct become inserted in a vector for expression, through the usage of the Snap gene tool (https://www.snapgene.com/free-trial/). A well-suited plasmid vector pET-28a (+) was used to integrate the optimized sequence and clone the built chimeric vaccine. pET-28a (+) is for expression of N-terminally 6 × His- tagged proteins and usually, N-terminaltags are effective over C-terminal tags, leading to more suitable purification, protein recovery, and more potent response (34).

Result

Strain Identification and Protein Sequence Retrieval

The total of eight protein sequences was selected for the possible vaccine construction. (Table 1).

Table 1. Lists the proteins sequences with their NCBI accession numbers

Name of protein	Accession Numbers
G	Q9IH62
FG	Q9IH63
M	Q9IK90
P	Q9IK91
N	NP_112021.1
L	Q997F0
V	NP_112023.1
W	YP_007188592.1

Prediction of Antigenicity and Physicochemical Properties of the Proteins

All selected Proteins from NiV were found to be antigenic. All the physicochemical properties support our constructed vaccine as promiscuous against Nipah Virus (Table 2). All of them had a predicted half-life of 30 h in mammalian reticulocytes and all of them were predicted to be stable. The **FG** had the highest predicted aliphatic index of 112.44, as well as the highest predicted extinction co- efficient of 76,620 M−1 cm− was **L** and the lowest GRAVY value was predicted for W Protein, all the other proteins from the selected viruses had almost similar theoretical Pi (Table 2).

Table2. Physiochemical properties of selected proteins

Protein	NO Amino Acids	Antigenicity	Molecular Weight (Daltons)	Theoretical PI	Aliphatic Index	GRAVY	Instability Index
G	602	0.5110	67039.03	8.58	90.95	-0.178	34.56
FG	546	0.5012	60281.96	5.85	112.44	0.195	38
M	352	0.4033	39928.28	9.31	90.26	-0.211	29.53
P	532	0.5713	58168.07	6.06	86.28	-0.236	52.33
N	709	0.5866	78302.51	4.6	76.29	-0.73	48.52
L	2244	0.4757	257232.51	7.53	94.57	-0.286	41.87
V	456	0.6252	50325.44	4.66	65.61	0.816	60.47
W	449	0.6199	49464.67	4.84	67.93	-0.827	57.56

T-cell and B-cell Epitope Prediction and IFN-gamma Induction Capacity

The T-cell and B-cell epitopes of the eight NiV Proteins have been predicted for vaccine construction. These epitopes have been predicted to stimulate the capacity T-cell and B-cell immune responses. Based on their rankings, the top MHC class-I and

MHC class-II epitopes in addition to B-cell epitopes with length over four amino acids, have been considered for all of the viral Proteins. From those epitopes, the antigenic, non-allergenic, nontoxic, 100% conserved and non- homologous epitopes have been taken into consideration because the exceptional selected epitopes and decided on for final vaccine construction. IFN-gamma inducing capacity prediction of the HTL epitopes had confirmed that maximum of the chosen HTL epitopes induce cytokine.

Prediction of B-Cell Epitopes

Predicted B-cell epitopes with BepiPred linear epitope with a threshold value of 0.35. Emini threshold value 1 and Kolaskar and Tongaonker threshold value 1. 025.This list was downsized by selecting epitopes sized between four to fifteen peptides. (Table 3).

Table 3. Predicted B cell epitopes from the proteins

Protein Name	Epitope	Emine Score(threshold=1.0)	Kolaskar and Tongaonker
G	VISRPGQSQ	1.0klsw47	1.044
FG	NNTHDLV QDYA	1.109 1.928	1.109 1.026
M	KSASHPQDL KSASHPQ KSASHP KSASH RAGKYYSVDY RAGKYYSVD RAGKYYSV VERTPETG PLNH EGVS	2.068 2.46 1.821 1.51 2.222 1.814 1.386 1.386 1.079 1.036	1.035 1.029 1.031 1.025 1.049 1.036 1.057 1.057 1.049 1.03
P	PQKRLPML PQKRLP PQKRL NPELKPV LEQQS LEQQ	1.082 2.342 2.008 1.072 1.333 1.324	1.034 1.033 1.026 1.045 1.029 1.033
N	RHHA KTARDSSKGKTPFVD	1.331 2.838	1.037 2.838
L	FDPY EWDSVYP EWDSVY KYFK	1.266 1.251 1.034 1.961	1.046 1.033 1.028 1.028

	RVPY	1.273	1.12
V	KDSAVK PQKRLPML PQKRLP PQKRL PAKDSPV PAKD QLDP	1.155 1.076 2.301 1.972 1.043 1.584 1.12	1.031 1.034 1.033 1.026 1.055 1.584 1.049
W	KDSAVK PQKRLP PAKDSPV QLDP	1.117 2.227 1.003 1.092	1.031 1.033 1.055 1.049

Predicted T cell epitopes from the proteins

The analysis of proteins for MHC-1epitopes using IEDB program showed that there were 6, 7, 6, 19, 13, 52, 6 and 7 predicted epitopes for G, FG, M, N, P, L, V and W proteins, respectively, with an IC_{50} from 1 to 100.selected MHC-I epitopes based on their antigenicity score and the number of reactions with different alleles (Table 4). Moving to MHC-II epitopes, there were6, 1, 15, 37, 12, 61, 9 and 6 predictions for G, FG, M, N, P, L, V and W proteins respectively, and these predictions were analyzed according to their antigenicity score, the number of reactions with different alleles, and their ability to induce interferon-gamma (Tables 5).

Table4.MHC I epitopes predicted for proteins studied.

Protein Name	MHC I	Protein Name	MHC I
G	IMIIQNYTR ITIPANIGL KCKFTLPPL SLIDTSSTI TEIGPKVSL TLYFPAVGF	**FG**	KIKSNPLTK KLSKIGLVK KTVYVLTAL KYKIKSNPL MLSMIILYV YINTNLVPT YVLTALQDY
M	MYLICYGFV TIAAYPLGV VSDFSPSSW YMYLICYGF YPLGVGKSA YTPGANERK	**N**	AVQETSAGR ETGMAGFFA FAMGVATTI FAPGGYPLL GPRAPYMVL GYLEPMYFR LLWSFAMGV PMYFRLGQK QQKRVNPFF QVAELAAAV RVNPFFALT SGRQSVTFK SLMLLYREI SSKGKTPFV TIRFGLETR TLVSAVITI VIIDVGSMV VQQKRVNPF YPLLWSFAM
P	DFSNTFFPH DPVVTDVVY EIAVSKEDR KGKGERKGK KLINLDMRL KSRGIPIKK MIMIPGKGK MPKSRGIPI QLDPVVTDV RLNYHADHL SLFSFDNVK TPMPKSRGI VLAKTNTAL	**L**	EVYLLCLQK FALYLGQSI FLDWASDPY FLFLSAYET FPLWSTEEL FSINETLTL GFNYLNLSR GQVDAELPI GYSQKTWTI IGLNSSSCY IPFLFLSAY IVDPELFAL IVYSLIKFK KDKALSPIK KLKGLVVPL KSRELDFPL KYRRIGLNS LASFLMDRR LIDPLFPVM LLISTEFSI LPYKVKKEI LSAYETNTR LSNREVKIW

			LVLALLQLK NIRRKVLIL NPDSTEVYL NTMYGWFFV RLRDKSTQF RLRMNLRAL RPTKALRSA RSNALWGLI SFLDYHTEF SISFYNSGI SMIEPLVLA SMMLLYQST SPISNNLDW SPNPMLKGL TASLADLKR TEFNPHNHY TIATIPFLF TQFKFAGSV TVAQTVLEI VGYCINILK VIFYASLTY VMGNRIYNI VTASLADLK WTIATIPFL YASLTYLRR YGLPGFFNW YLLCLQKTV YPKGGIEGY YVGSSTDER
V	DPVVTDVVY EIAVSKEDR MPKSRGIPI QLDPVVTDV TPMPKSRGI SSSEVIVGI	**W**	DPVVTDVVY EIAVSKEDR KSRGIPIKK MPKSRGIPI QLDPVVTDV TPMPKSRGI SSSEVIVGI

Table5.MHC II epitopes predicted for proteins studied.

Protein Name	**MHC II epitope**	**Protein Name**	**MHC II eoitope**
G	GLLGSKISQ IIGVGEVLD IIVMNIMII LYFPAVGFL TITIPANIG TLYFPAVGF	**FG**	KTVYVLTAL

M	AGKYYSVDY AYPLGVGKS EISRVAAVL ISRVAAVLQ LNHLVPWKK NHLVPWKKV PGANERKYN RRTAGSTEK RTMLEFRRN RTPETGKRK RVAAVLQPS SRVAAVLQP VSDFSPSSW YDDVFIDNT YMYLICYGF	**N**	AELAAAVQE AKGRAVEII ALNINRGYL AVIIDVGSM AVITIEAQI DIEAVIIDV EAVIIDVGS EIGPRAPYM ELAAAVQET EPMYFRLGQ FAPGGYPLL GRASAATAT GYLEPMYFR GYPLLWSFA IEAVIIDVG IGPRAPYMV IKSLMLLYR INRGYLEPM KFAPGGYPL KGRAVEIIS KKGGSAKGR LETRYPALA LLWSFAMGV LMLLYREIG NINRGYLEP NRGYLEPMY QQKRVNPFF QVAELAAAV RVNPFFALT RYPALALNE SAKGRAVEI VAELAAAVQ VIIDVGSMV VLIGGSDQD VQQKRVNPF VSAVITIEA WILIAKAVT

P	DFSNTFFPH DQLEFEDEF EIAVSKEDR GKGKGERKG GTPMPKSRG INSIKLINL LIRTHIKDR MIMIPGKGK PMPKSRGIP SRGIPIKKG TLIRTHIKD VSNTRDWAE	**L**	AALIPAPIG ATIPFLFLS DEDLELASF DIKYQPLIS DPELFALYL DVLEGRMMM EQGNTMYGW EQGNTMYGW EVYLLCLQK FCLNWRYES FEIHHRRPR FERLRMNLR FGHPILEAK FLDWASDPY GDASFLDWA HIIRLKNSG IEPLVLALL IGLNSSSCY IKLGNVKRP ILDFRSKLM ILNIDNIHL IVDPELFAL KTWTIATIP KYRRIGLNS LCVIIDLYA LDWLITAAL LIDPLFPVM LLCLQKTVK LLSILNIDN LNIDNIHLL LRIRQVTEV LVLALLQLK MGNRIYNIV NFDPYNMLE NISTTIAKA NTMYGWFFV NTRIAAIVQ PAPIGGFNY PFLFLSAYE PISNNLDWL PSEYSIAEE QKIRSMFID QQLLISTEF RLRDKSTQF RLRMNLRAL RSNALWGLI SFLDWASDP SFLDYHTEF SFLMDRRVI SYFGLVLVC TAGRSIGLV TIATIPFLF TLGQSISFY TVAQTVLEI

			TWTIATIPF VMGNRIYNI VYLLCLQKT WGLIDPLFP WKIIGYISI WTIATIPFL WWKIIGYIS YHTEFNPHN YVGSSTDER
V	DLSSTSPTD DQLEFEDEF GTPMPKSRG IQKNQKEIQ KSRGIPIKK LSSTSPTDG MPKSRGIPI SSSEVIVGI VSNTRDWAE	**W**	DQLEFEDEF EEADQLEFE IQKNQKEIQ PKSRGIPIK SSEVIVGIS SSSEVIVGI

D Structure Generation

The final 3D model structure of the vaccine construct is shown in (Figure1). The good results with N, G, FG and M were found by the Ramachandran plot (Figure2).

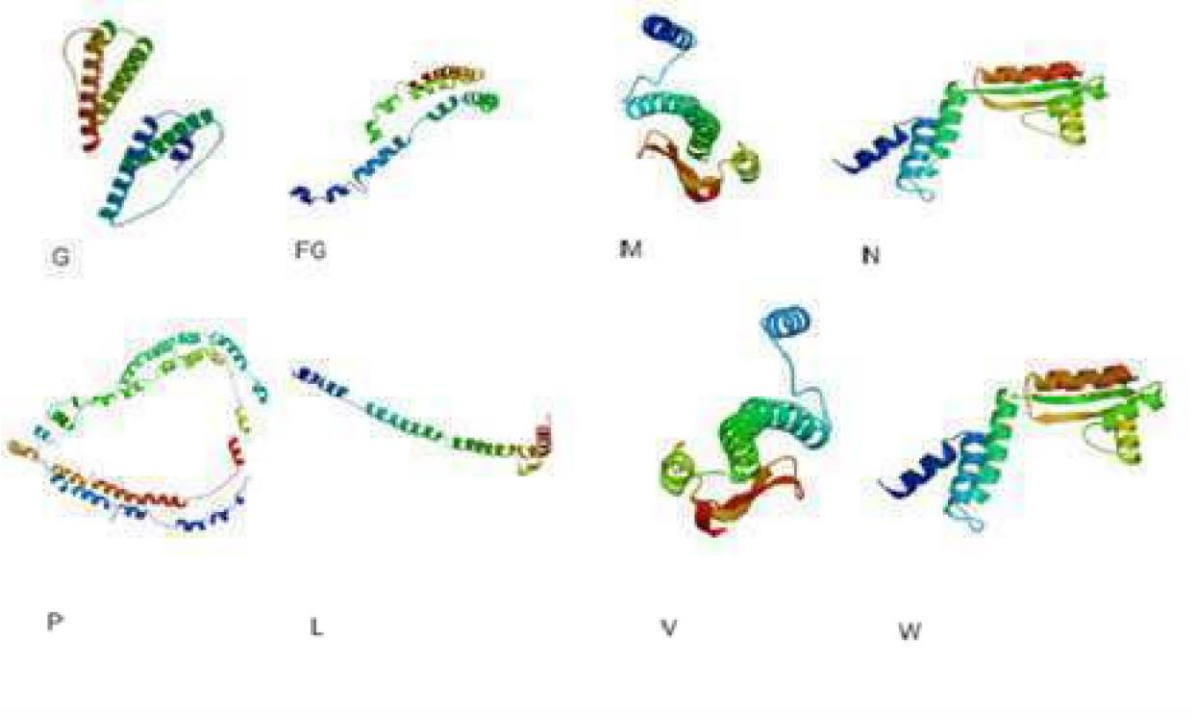
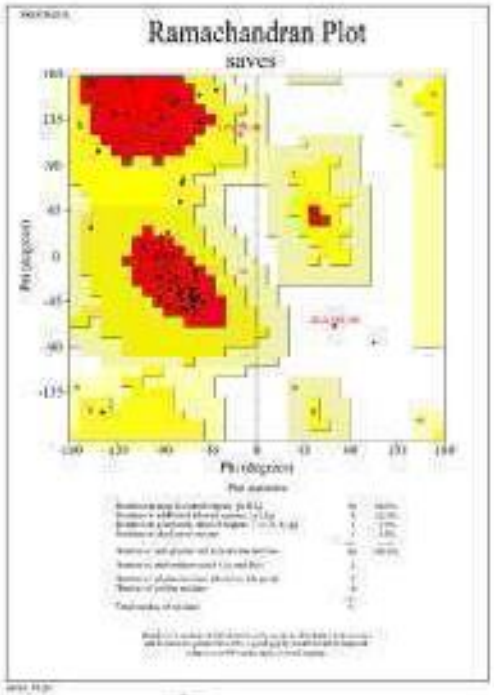
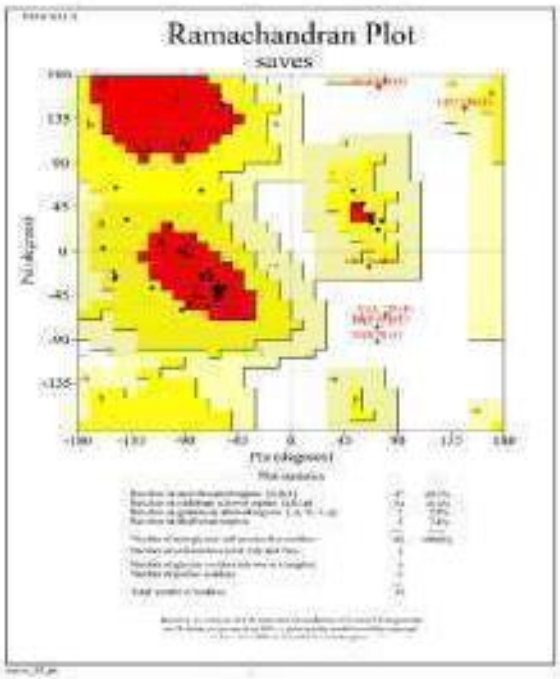


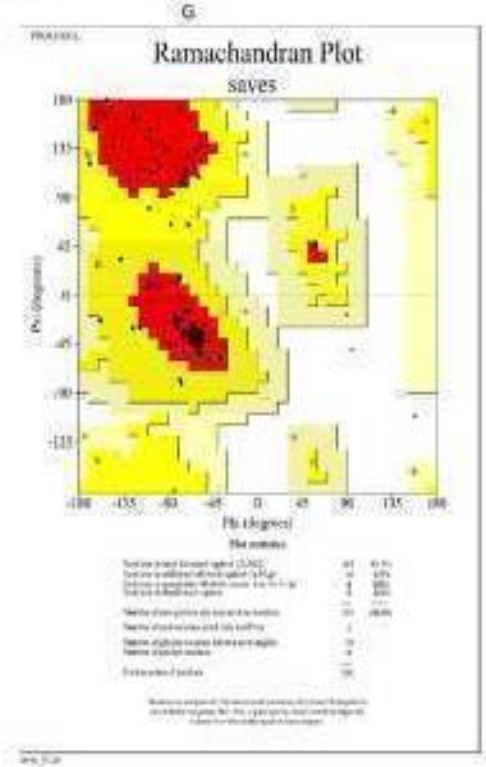
Figure 1. 3D structural of final Vaccines


Ramachandran Plot
saves

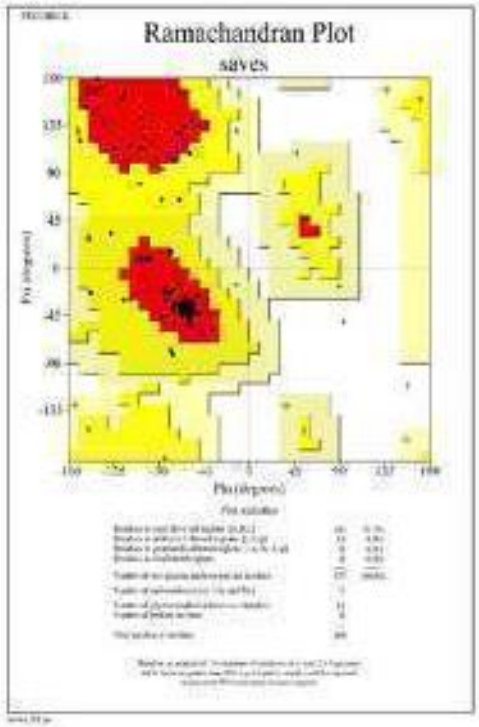
G


Ramachandran Plot
saves

FG


Ramachandran Plot
saves

M


Ramachandran Plot
saves

P

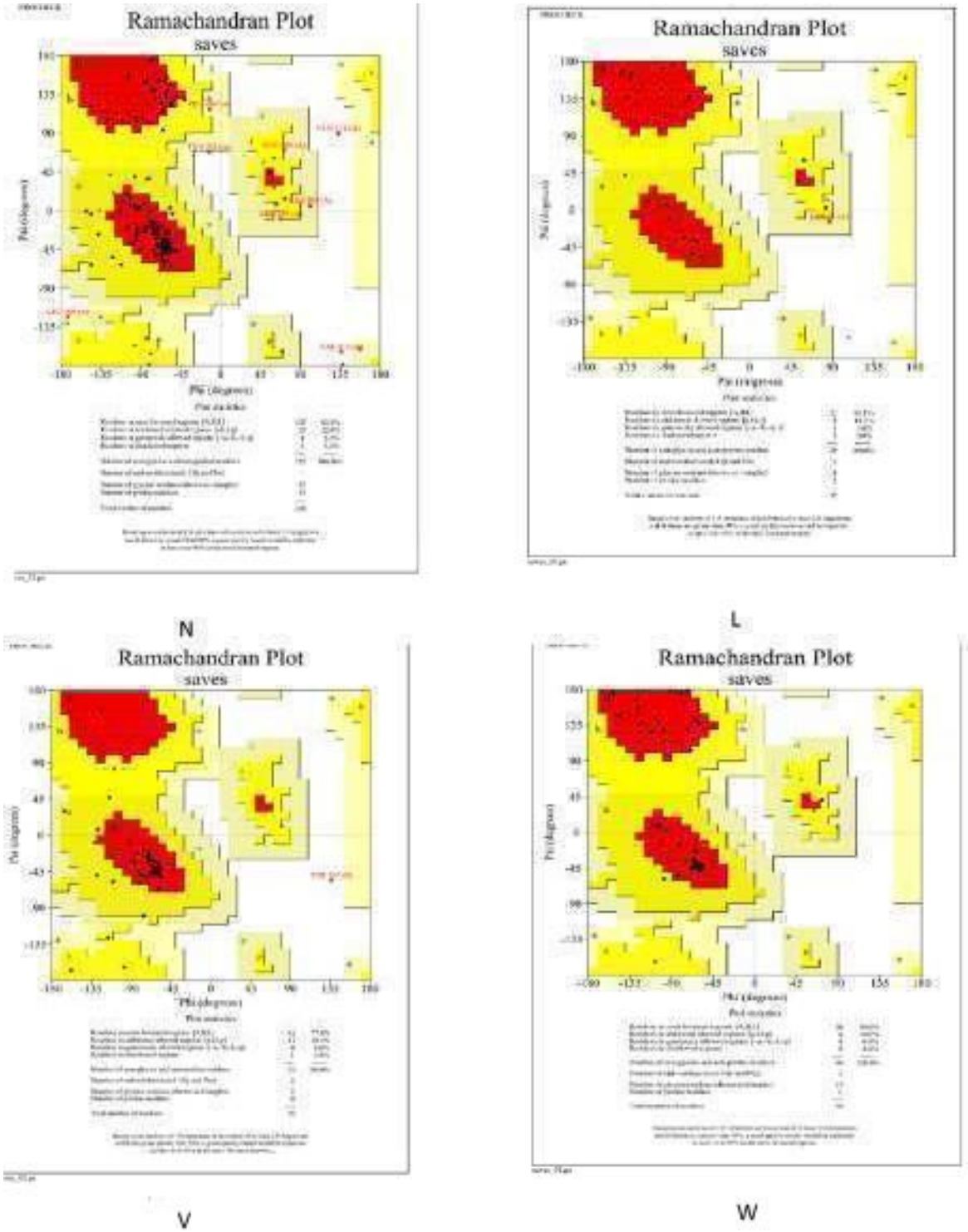


Figure 2. Ramachandran validation plot, for all constructed vaccine

Vaccine Construction

Eight different constructs were built by the integration of antigenic peptides and several adjuvants and linkers. There were a lot of possible arrangements of segments but since the analysis of such a huge number of constructs were impossible. This study designed these eight constructs as shown in (Table 6) as samples for this study. The sequence of these

constructs differed from each other according to the adjuvant used and also the arrangement order of the constituent segments what about the other constructs you have to show the criteria very clearly. The linkers "EAAAK" (Orange) links the adjuvant (black) in the constructs. The GGGGS (Light blue) and GG (Light blue) link the MHC I epitopes. GPGPG (Purple) and KK(Purple) link the MHC II epitopes. KK (Dark blue) and AAY (Dark blue) link B-Cell epitopes. MHC I epitopes (Green), MHC II (Moss), the immune enhancer adjuvant as well as linker sequences were inserted as shown in (Table 6).

Table 6. Vaccines Constructs. An adjuvant in Black, Linker "EAAAK" joining adjuvant shown in Orange, linkers "GGGGS""GG" joining epitopes shown in Dark Blue, epitopes shown in Green and PADER sequence shown in Moss.

G adjuvant = HBHA adjuvant
EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQFIELRDKFTTEE LRKAAEGYLEAATNRYNELVERGEAALQRLRSQTAFEDASARAEGYVDQAVELTQEALGTVASQTRAVGERAAKLVGIELPGKA EAAGKKAQKAIAKAPAKKASAKKAPAKKAPAKKAAAKKVTQKGGGGSIMIIQNYTRGGGGSITIPANIGLGGGGSKCKFTLPPL GGGGSSLIDTSSTIGGGGSTEIGPKVSLGGGGSTLYFPAVGFGPGPGGLLGSKISQGPGPGIIGVGEVLDGPGPGIIVMNIMII GPGPGLYFPAVGFLGPGPGTITIPANIGGPGPGTLYFPAVGFKKVISRPGQSQEAAAK
FG adjuvant = HBHA adjuvant
EAAAKMAENSNIDDIKAPLLAALGAADLALATVNELITNLRERAEETRRSRVEESRARLTKLQEDLPEQLTELR EKFTAEELRKAAEGYLEAATSELVERGEAALERLRSQQSFEEVSARAEGYVDQAVELTQEALGTVASQVEGR AAKLVGIELGGGGSKIKSNPLTKGGGGSKTVYVLTALGGGGSKYKIKSNPLGGGGSMLSMIILYVGGGGSYIN TNLVPTGGGGSYVLTALQDYGPGPGKTVYVLTALKKNNTHDLVKKQDYAEAAAK
M adjuvant = flagellin adjuvant
EAAAKMAQVINTNSLSLLTQNNLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNAND GISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQRLEEIDRVSNQTQFNGVKVLSQDNQM KIQVGANDGETITIDLQKIDVKSLGLDGFNVGGGGSMYLICYGFVGGGGSTIAAYPLGVGGGGSVSDFSPSSW GGGGSYMYLICYGFGGGGSYPLGVGKSAGGGGSYTPGANERKGPGPGAGKYYSVDYGPGPGAYPLGVGKSG PGPGEISRVAAVLGPGPGISRVAAVLQGPGPGLNHLVPWKKGPGPGNHLVPWKKVGPGPGPGANERKYNGP GPGRRTAGSTEKGPGPGRTMLEFRRNGPGPGRTPETGKRKGPGPGRVAAVLQPSGPGPGSRVAAVLQPGPGP GVSDFSPSSWGPGPGYDDVFIDNTGPGPGYMYLICYGFKKKSASHPQDLKKKSASHPQKKKSASHPKKKSASH KKRAGKYYSVDYKKRAGKYYSVDKKRAGKYYSVKKVERTPETGKKPLNHKKEGVSEAAAK
N adjuvant = Ribosomal protein adjuvant
EAAAKMAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILE AAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKGGGGSAVQET SAGRGGGGSETGMAGFFAGGGGSFAMGVATTIGGGGSFAPGGYPLLGGGGSGPRAPYMVLGGGGSGYLEP MYFRGGGGSLLWSFAMGVGGGGSPMYFRLGQKGGGGSQQKRVNPFFGGGGSQVAELAAAVGGGGSRVNPF FALTGGGGSSGRQSVTFKGGGGSSLMLLYREIGGGGSSSKGKTPFVGGGGSTIRFGLETRGGGGSTLVSAVITI GGGGSVIIDVGSMVGGGGSVQQKRVNPFGGGGSYPLLWSFAMGPGPGAELAAAVQEGPGPGAKGRAVEIIGP GPGALNINRGYLGPGPGAVIIDVGSMGPGPGAVITIEAQIGPGPGDIEAVIIDVGPGPGEAVIIDVGSGPGPGEIG PRAPYMGPGPGELAAAVQETGPGPGEPMYFRLGQGPGPGFAPGGYPLLGPGPGGRASAATATGPGPGGYLE PMYFRGPGPGGYPLLWSFAGPGPGIEAVIIDVGGPGPGIGPRAPYMVGPGPGIKSLMLLYRGPGPGINRGYLE PMGPGPGKFAPGGYPLGPGPGKGRAVEIISGPGPGKKGGSAKGRGPGPGLETRYPALAGPGPGLLWSFAMG VGPGPGLMLLYREIGGPGPGNINRGYLEPGPGPGNRGYLEPMYGPGPGQQKRVNPFFGPGPGQVAELAAAV GPGPGRVNPFFALTGPGPGRYPALALNEGPGPGSAKGRAVEIGPGPGVAELAAAVQGPGPGVIIDVGSMVGPG PGVLIGGSDQDGPGPGVQQKRVNPFGPGPGVSAVITIEAGPGPGWILIAKAVTKKRHHAKKKTARDSSKGKTP FVDEAAAK
P adjuvant = HBHA adjuvant
EAAAKMAENPNIDDLPAPLLAALGAADLALATVNDLIANLRERAEETRAETRTRVEERRARLTKFQEDLPEQF IELRDKFTTEELRKAAEGYLEAATNRYNELVERGEAALQRLRSQTAFEDASARAEGYVDQAVELTQEALGTV ASQTRAVGERAAKLVGIELGGGGSDFSNTFFPHGGGGSDPVVTDVVYGGGGSEIAVSKEDRGGGGSKGKGER KGKGGGGSKLINLDMRLGGGGSKSRGIPIKKGGGGSMIMIPGKGKGGGGSMPKSRGIPIGGGGSQLDPVVTD VGGGGSRLNHIEEQVGGGGSRLNYHADHLGGGGSSLFSFDNVKGGGGSTPMPKSRGIGGGGSVLAKTNTALG PGPGDFSNTFFPHGPGPGDQLEFEDEFGPGPGEIAVSKEDRGPGPGGKGKGERKGGPGPGGTPMPKSRGGPG PGINSIKLINLGPGPGLIRTHIKDRGPGPGMIMIPGKGKGPGPGPMPKSRGIPGPGPGSRGIPIKKGGPGPGTLI RTHIKDGPGPGVSNTRDWAEKKPQKRLPMLKKPQKRLPKKPQKRLKKNPELKPVKKLEQQSKKLEQQEAA AK
V adjuvant = flagellin adjuvant

EAAAKMAQVINTNSLSLLTQNNLNKSQSSLSSAIERLSSGLRINSAKDDAAGQAIANRFTSNIKGLTQASRNAND GISIAQTTEGALNEINNNLQRVRELSVQATNGTNSDSDLKSIQDEIQQRLEEIDRVSNQTQFNGVKVLSQDNQM KIQVGANDGETITIDLQKIDVKSLGLDGFNVGGGGSDPVVTDVVYGGGGSEIAVSKEDRGGGGSMPKSRGIPIG GGGSQLDPVVTDVGGGGSSSSEVIVGIGGGGSTPMPKSRGIGPGPGDLSSTSPTDGPGPGDQLEFEDEFGPGPG GTPMPKSRGGPGPGIQKNQKEIQGPGPGKSRGIPIKKGPGPGLSSTSPTDGGPGPGMPKSRGIPIGPGPGSSSEV IVGIGPGPGVSNTRDWAEKKKDSAVKKKPQKRLPMLKKPQKRLPKKPQKRLKKPAKDSPVKKPAKDKKQLD PEAAAK
W adjuvant = Ribosomal protein adjuvant
EAAAKMAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAEEQSEFDVILE AAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAKAKLEAAGATVTVKGGGGSDPVVT DVVYGGGGSEIAVSKEDRGGGGSKSRGIPIKKGGGGSMPKSRGIPIGGGGSQLDPVVTDVGGGGSSSSEVIVGI GGGGSTPMPKSRGIGPGPGDQLEFEDEFGPGPGEEADQLEFEGPGPGIQKNQKEIQGPGPGPKSRGIPIKGPGP GSSEVIVGISGPGPGSSSEVIVGIKKKDSAVKKKPQKRLPKKPAKDSPVKKQLDPEAAAK
L adjuvant = Beta defensin adjuvant
EAAAKGIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKKGGEVYLLCLQKGGSFALYLGQS IGGFLDWASDPYGGFLFLSAYETGGFPLWSTEELGGFSINETLTLGGGFNYLNLSRGGGQVDAELPIGGGYSQ KTWTIGGIGLNSSSCYGGIPFLFLSAYGGIVDPELFALGGIVYSLIKFKGGKDKALSPIKGGKLKGLVVPLGGKS RELDFPLGGKYRRIGLNSGGLASFLMDRRGGLIDPLFPVMGGLLISTEFSIGGLPYKVKKEIGGGGSLSAYETN TRGGGGSLSNREVKIWGGLVLALLQLKGGNIRRKVLILGGNPDSTEVYLGGNTMYGWFFVGGRLRDKSTQF GGRLRMNLRALGGRPTKALRSAGGRSNALWGLIGGRTAGRSIGLGGSFLDYHTEFGGSISFYNSGIGGSMIEP LVLAGGSMMLLYQSTGGSPISNNLDWGGSPNPMLKGLGGTASLADLKRGGTEFNPHNHYGGTIATIPFLFGG TQFKFAGSVGGTVAQTVLEIGGVGYCINILKGGVIFYASLTYGGVMGNRIYNIGGVTASLADLKGGWTIATIPF LGGYASLTYLRRGGYGLPGFFNWGGYLLCLQKTVGGYPKGGIEGYGGYVGSSTDERKKAALIPAPIGKKATI PFLFLSKKDEDLELASFKKDIKYQPLISKKDPELFALYLKKDVLEGRMMMKKEQGNTMYGWKKEVYLLCLQ KKKFCLNWRYESKKFEIHHRRPRKKFERLRMNLRKKFGHPILEAKKKFLDWASDPYKKGDASFLDWAKKHI IRLKNSGKKIEPLVLALLKKIGLNSSSCYKKIKLGNVKRPKKILDFRSKLMKKILNIDNIHLKKIVDPELFALKK KTWTIATIPKKKYRRIGLNSKKLCVIIDLYAKKLDWLITAALKKLIDPLFPVMKKLLCLQKTVKKKLLSILNID NKKLNIDNIHLLKKLRIRQVTEVKKLVLALLQLKKKMGNRIYNIVKKNFDPYNMLEKKNISTTIAKAKKNTM YGWFFVKKNTRIAAIVQKKPAPIGGFNYKKPFLFLSAYEKKPISNNLDWLKKPSEYSIAEEKKQKIRSMFIDKK QQLLISTEFKKRLRDKSTQFKKRLRMNLRALKKRSNALWGLIKKSFLDWASDPKKSFLDYHTEFKKSFLMDR RVIKKSYFGLVLVCKKTAGRSIGLVKKTIATIPFLFKKTLGQSISFYKKTVAQTVLEIKKTWTIATIPFKKVMG NRIYNIKKVYLLCLQKTKKWGLIDPLFPKKWKIIGYISIKKWTIATIPFLKKWWKIIGYISKKYHTEFNPHNKK YVGSSTDERAAYFDPYAAYEWDSVYPAAYEWDSVYAAYKYFKAAYRVPYEAAAK

Antigenicity, Allergenicity, Toxicity, Solubility and Physicochemical Property Analysis

All the five vaccine constructs were checked for allergenicity, antigenicity, toxicity and solubility respectively as shown in (Table 7).Physiochemical properties of all vaccine constructs were shown in (Table 8).

Table7. Antigenicity, allergenicity, toxicity and solubility of the various vaccine constructs.

Vaccine Name	Antigenicity (Threshold > 0.4)	Allergenicity	Toxicity	Solubility
G	0.4106	Non-allergen	Non-toxin	**0.949833**
FG	0.5405	Non-allergen	Non-toxin	**0.956971**
M	0.5337	Non-allergen	Non-toxin	**0.846783**
N	0.5812	Non-allergen	Non-toxin	**0.998022**
P	0.6222	Non-allergen	Non-toxin	**0.986572**
L	0.5924	Non-allergen	Non-toxin	**0.900768**
V	0.5003	Non-allergen	Non-toxin	**0.967548**
W	**0.5029**	**Non-allergen**	**Non-toxin**	**0.936829**

Table 8. Physiochemical properties for all Vaccines constructed.

Vaccine Name	Number of Amino Acids	Molecular Weight (Daltons)	Theoretical pI	Aliphati-c Index	GRAVY	Instability Index
G	394	40011.73	9.42	84.14	-0.171	**42.49**
FG	273	28701.37	5.56	90.51	-0.319	**44.45**
M	569	58852.03	9.85	61.90	-0.643	**31.33**
N	947	91802.52	9.08	72.16	-0.034	**32.20**
P	580	59543.35	9.82	64.29	-0.692	**37.93**
L	1367	152684.17	10.06	91.05	-0.213	**35.56**
V	449	45963.49	9.60	68.62	-0.710	**38.84**
W	**353**	**35190.89**	**5.30**	**77.39**	**-0.328**	**34.27**

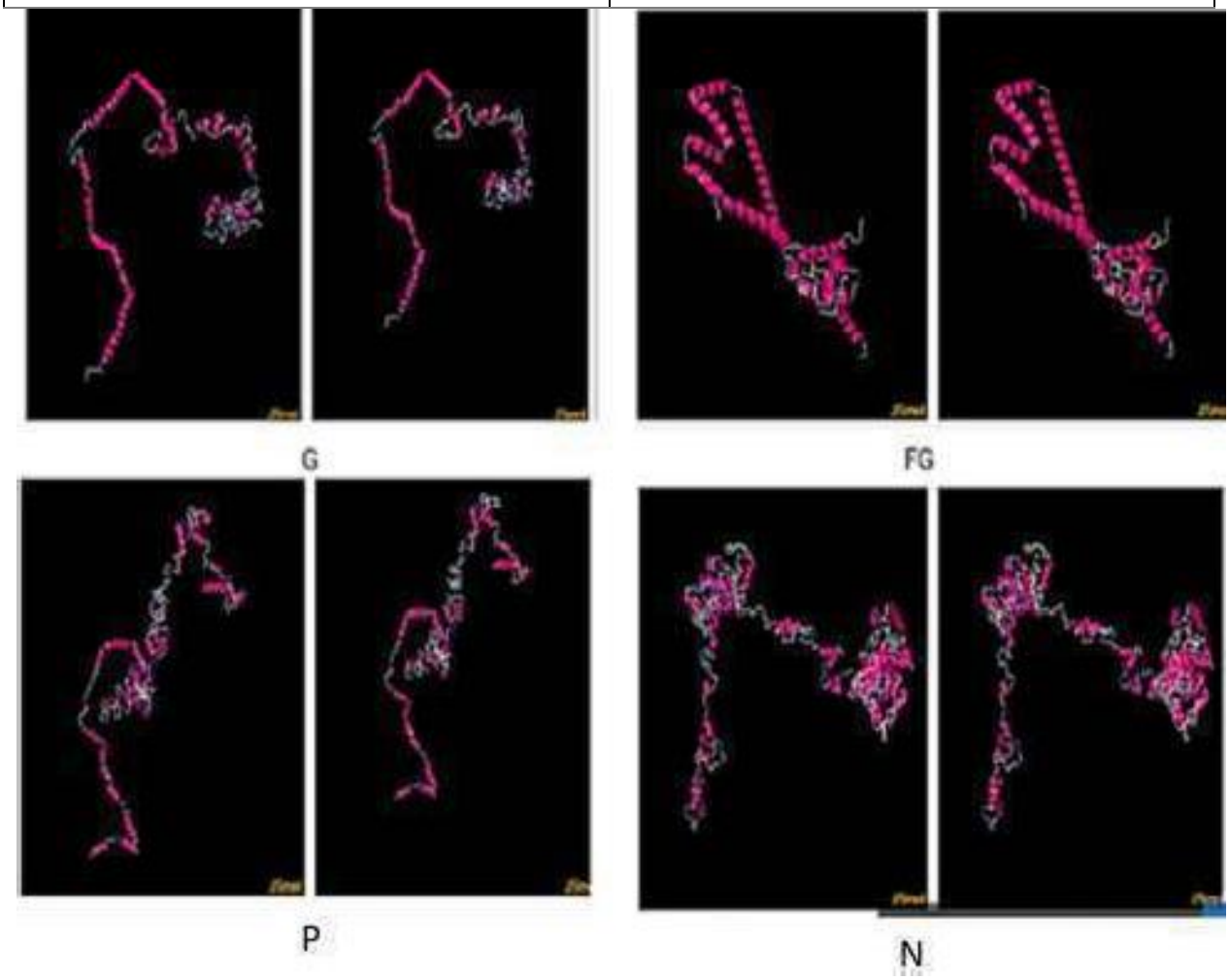
Vaccine Protein Disulfide Engineering

The Vaccine Constructs of G, FG, M, P, N, L, V and W were generated from32, 18, 51, 60, 105, 5, 32 and 38 viable pairs of amino acids, respectively the result shown in (Table 9). Then the disulfide bonds formation with the aid mutation were selected. The result shown the N protein was found to be the most constructed vaccine generating the highest number of disulfide bonds; this makes it stable among the 8 different vaccine constructs (Figure3).

Table 9. Disulfide bonds predicted for studied vaccine constructed.

Vaccine Name	Disulfide bond generated
G	97THR-100TYR, 165PRO-178ALA, 232ILE-235ASN, 275GLY-286THR and 328ILE-331MET
FG	4ALA-7ALA,192ILE-195ASN,202SER-244GLY,205SER-247VAL and 209LEU-228GLY
M	2ALA-14SER,163ILE-490ALA,218SER-221SER,311GLY-322PRO,347LYS-359ARG and 465GLY-468TYR
P	183SER-210GLY,237GLY-310ARG,285PRO-288THR,295SER-298ASN,316ASP-320GLY,325LEU-329,346ILE-350GLY,360LEU-380ASP,384PHE-387GLU,397VAL-400GLU,401-ASP-417GLY,414ARG-421GLY and 481LYS-490PRO
N	45VAL-48ALA,172GLY-230ALA,182SER-261PHE,239PRO-318GLY,280SER-283ASA,435ALA-438ILE,497VAL-502GLY,565ALA-568ALA,595SER-622PRO,634LEU-681SER,639ARG-

	646ASA,689GLY-694GLY,712GLY-730MET,785GLN-790ALA and 905ALA-924HIS
L	--
V	26SER-443ASP,199SER-231PRO and 204SER-207ASP
W	244GLU-279PRO,264GLY-267ILE,327LYS-348PRO and 253GLU-280GLY



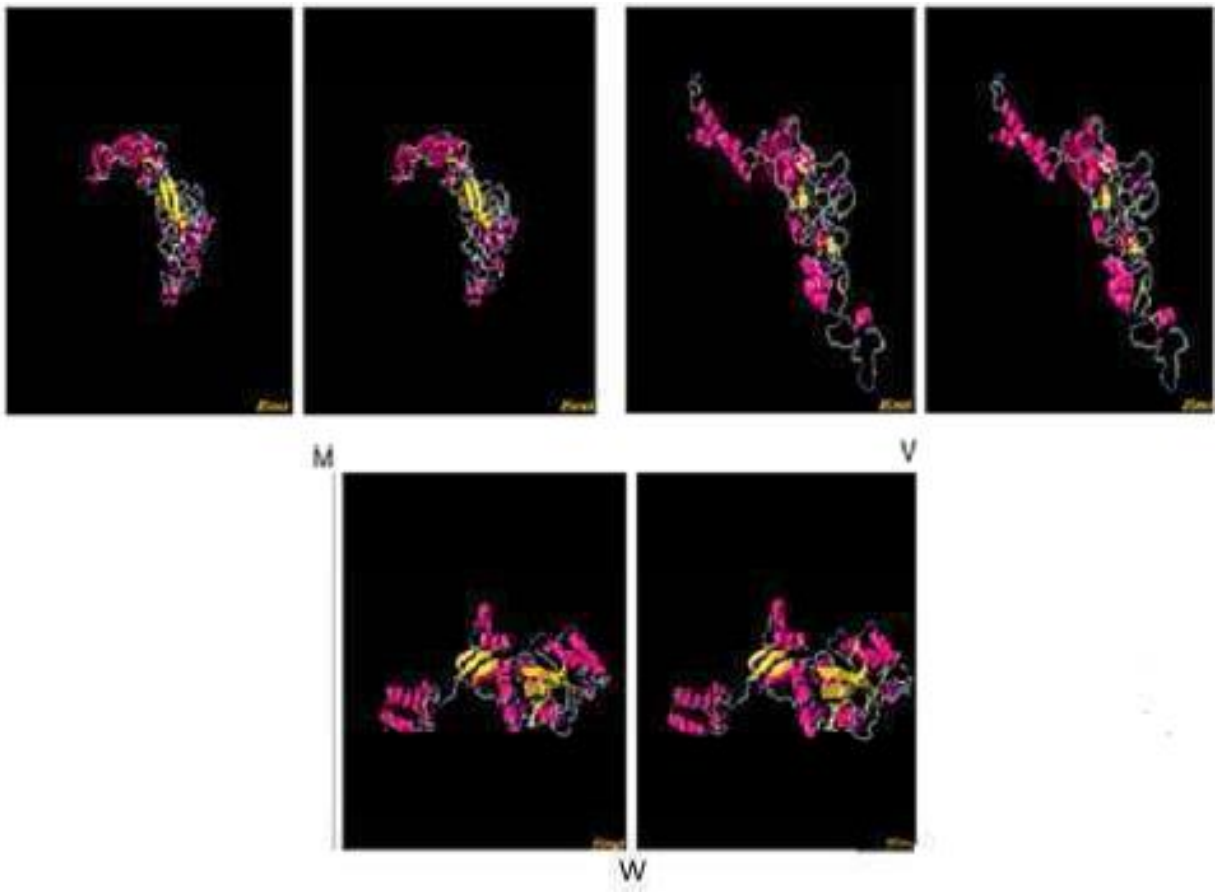


Figure 3. Disulfide bond identification in all vaccine constructed, except L protein there is no disulfide bond identification(Yellow color indicate disulfide bond generation).

Molecular Docking Analysis

The result of vaccine constructs against MHC allele (PDB ID: 4U6X) TLR8 in complex with Resiquimod crystal form (TLR8 PDB ID: 3W3M), Crystal structure of human TLR8 unliganded form (TLR8 PDB ID: 3W3G) and (TLR PDB ID: 6SWS) were shown in (Table 10).

Table 10. Docking result from chosen server and Ligands.

Vaccine name	PDB ID target	ClusPro energy Score	HawkDock score	MM-GBSA (binding free energy,inKal mol^{-1})
G	3W3M	-1244.(	-5131.86	-45.09
	3SWS	-1088.9	-3867.94	-7.79
	4U6X	-903.6	-4895.98	-6.77
FG	3W3M	-1081.4	-5919.20	-18.49
	3SWS	-905.3	-2306.21	-3.86

	4U6X	-711.4	-4596.04	21.44
M	3W3M	1103.1	-6163.86	42.22
	3SWS	1084.0	-5146.20	20.68
	4U6X	-825.9	-5191.14	
P	3W3M	1286.5	-5435.17	26.14
	3SWS	1326.9	-4526.94	-7.9
	4U6X	-874.4	-5250.38	50.96
N	3W3M	1600.6	-5148.12	26.88
	3SWS	1135.0	-3842.46	-45.5
	4U6X	-934.0	-5469.07	14.83
L	3W3M	-903.0	-4477.73	34.29
	3SWS	-923.1	-5009.79	-75.8
	4U6X	-659.1	-4340.12	44.52
V	3W3M	-912.4	-6434.07	-3.55
	3SWS	---	-3151.04	11.21
	4U6X	-817.3	-4431.82	0.32
W	3W3M	-741.4	-3865.94	-6.07
	3SWS	-738.0	-3515.02	11.12
	4U6X	-506.9	-3707.36	30.92

Screening for Conformational B-lymphocytic Epitopes

The results of Conformational B-lymphocytic epitopes for the proteins were shown in (Table 11).

Table11. Discontinues B-lymphocyte for constructed vaccines.

Vaccine Name	Total residue found	Score Variation	Length of peptide
G	26	0.579 to 0.894	4 to 10
FG	28	0.559 to 0.892	3 to 18
M	65	0.594 to 0.79	3 to 31
P	38	0.566 to 0.783	3 to 16
N	102	0.547 to 0.774	3 to 40
L	17	0.631 to 0.8	4 to 13
V	28	0.514 to 0.77	3 to 11
W	29	0.53 to 0.843	7 to 12

Molecular Dynamics Simulation

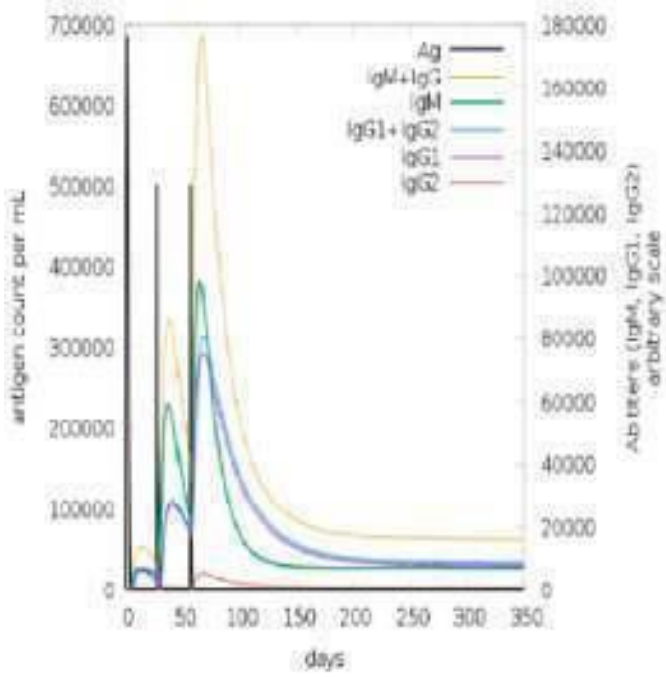
The result of the molecular simulation of proteins showed in (Table 12) and (Figure 9).

Table12. Molecular dynamics simulation of the predicted vaccine–TLR3 complex.

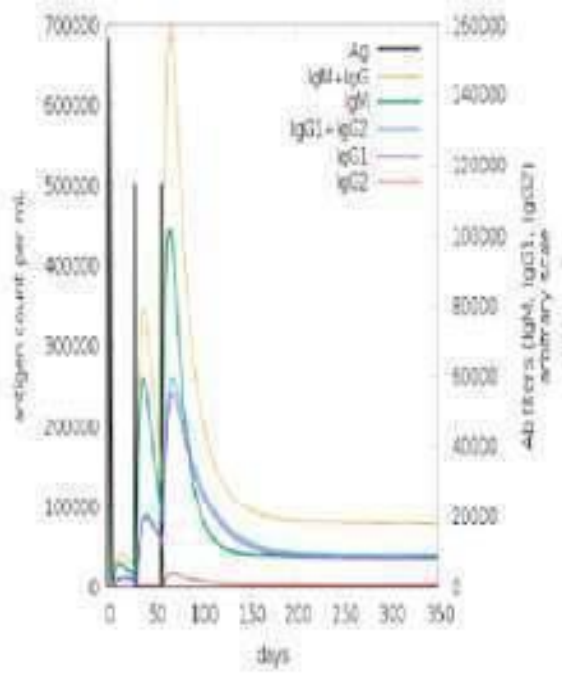
Vaccine name	High level of fluctuation predicted	Temperature for each fluctuation
G	(350,760,818,142,81,77),	(3.9 A^o ,3.6 A^o,5.8 A^o , 5.6 A^o , 3.4 A^o , 3.1 A^o)
FG	(818, 571, 628, 141,138),	(5.0 A^o , 4.1 A^o , 3.7 A^o , 9.1 A^o , 8.4 A^o)
M	(10,22,50),	(3.8 A^o , 3.5 A^o , 2.7 A^o)
P	(335,256,526,333),	(4.7 A^o , 3.4 A^o , 3.9 A^o , 1.9 A^o)
N	(446,410,408,425,282),	(5.3 A^o , 5.0 A^o , 5.0 A^o , 5.0 A^o , 4.9 A^o)
L	---	---
V	(31,263,760,135,108),	(3.2 A^o , 2.7 A^o , 2.7 A^o , 3.4 A^o , 2.9 A^o)
W	(196,1,41)	(4.7 A^o , 3.5 A^o , 2.8 A^o) ,

Immune Simulation

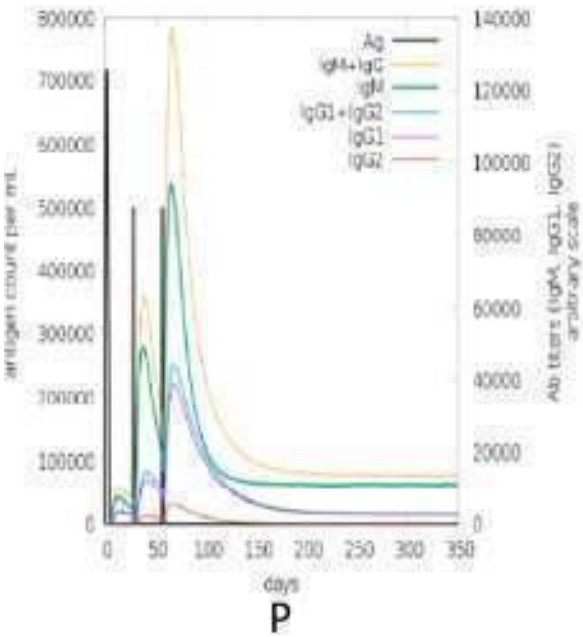
The immune simulation of these proteins confirmed that with the predicted capability of producing high levels of immunoglobulins, active B-cells and T-cell, cytokines and APCs, the constructed vaccines are probably capable of providing a good immunogenic protection in contrast to NiV (Figure 4,5,6,7).


Ag
IgM+IgG
IgM
IgG1+IgG2
IgG1
IgG2
antigen count per mL
Ab titers (IgM, IgG1, IgG2) arbitrary scale
days

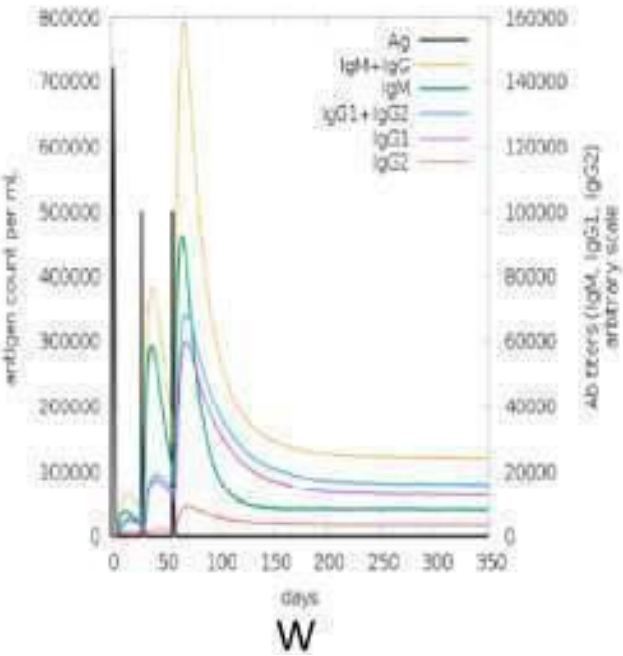
FG


Ag
IgM+IgG
IgM
IgG1+IgG2
IgG1
IgG2
antigen count per mL
Ab titers (IgM, IgG1, IgG2) arbitrary scale
days

G


Ag
IgM+IgG
IgM
IgG1+IgG2
IgG1
IgG2
antigen count per mL
Ab titers (IgM, IgG1, IgG2) arbitrary scale
days

P


Ag
IgM+IgG
IgM
IgG1+IgG2
IgG1
IgG2
antigen count per mL
Ab titers (IgM, IgG1, IgG2) arbitrary scale
days

W

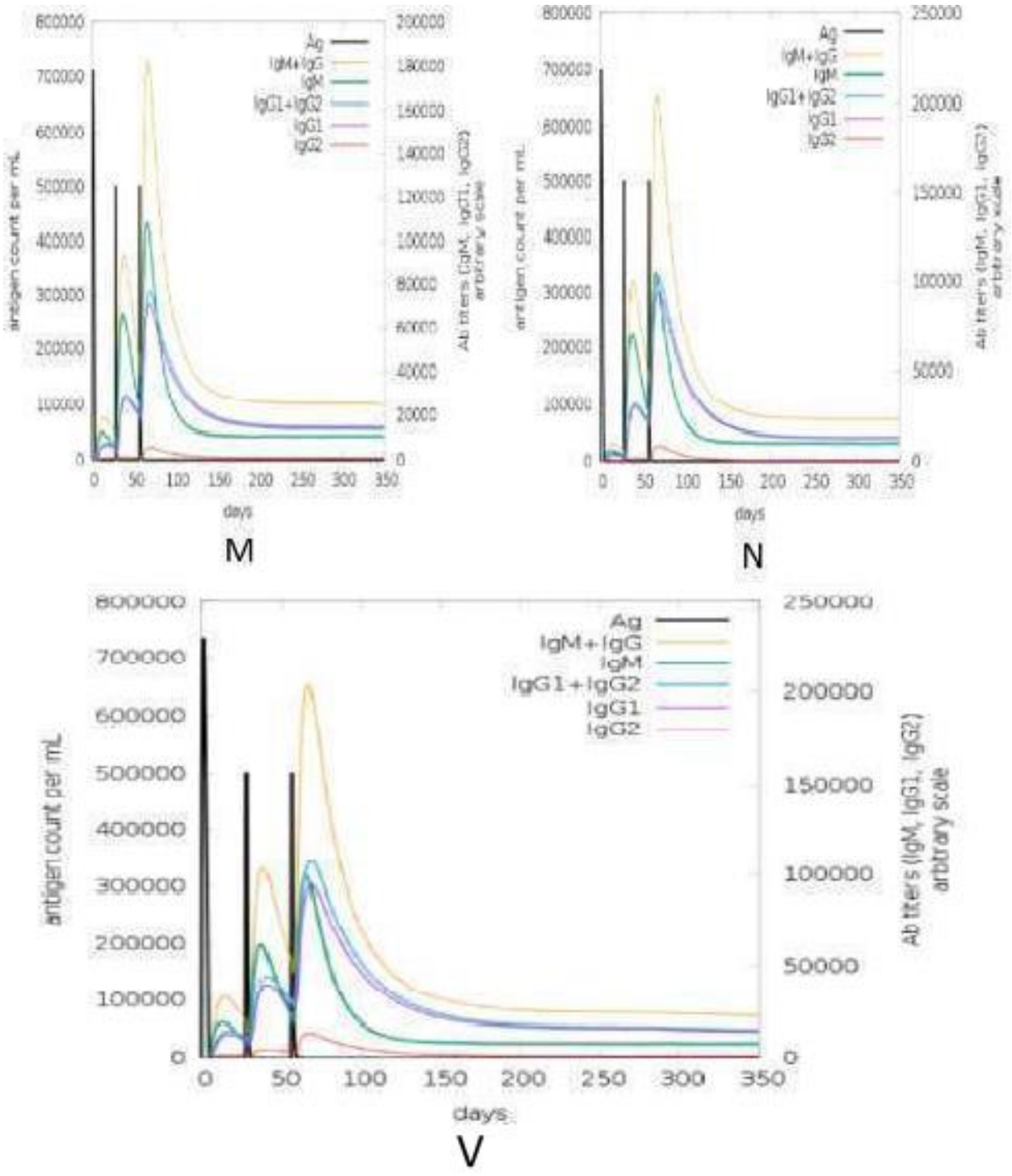


Figure 4. Antigen and immunoglobulins of control

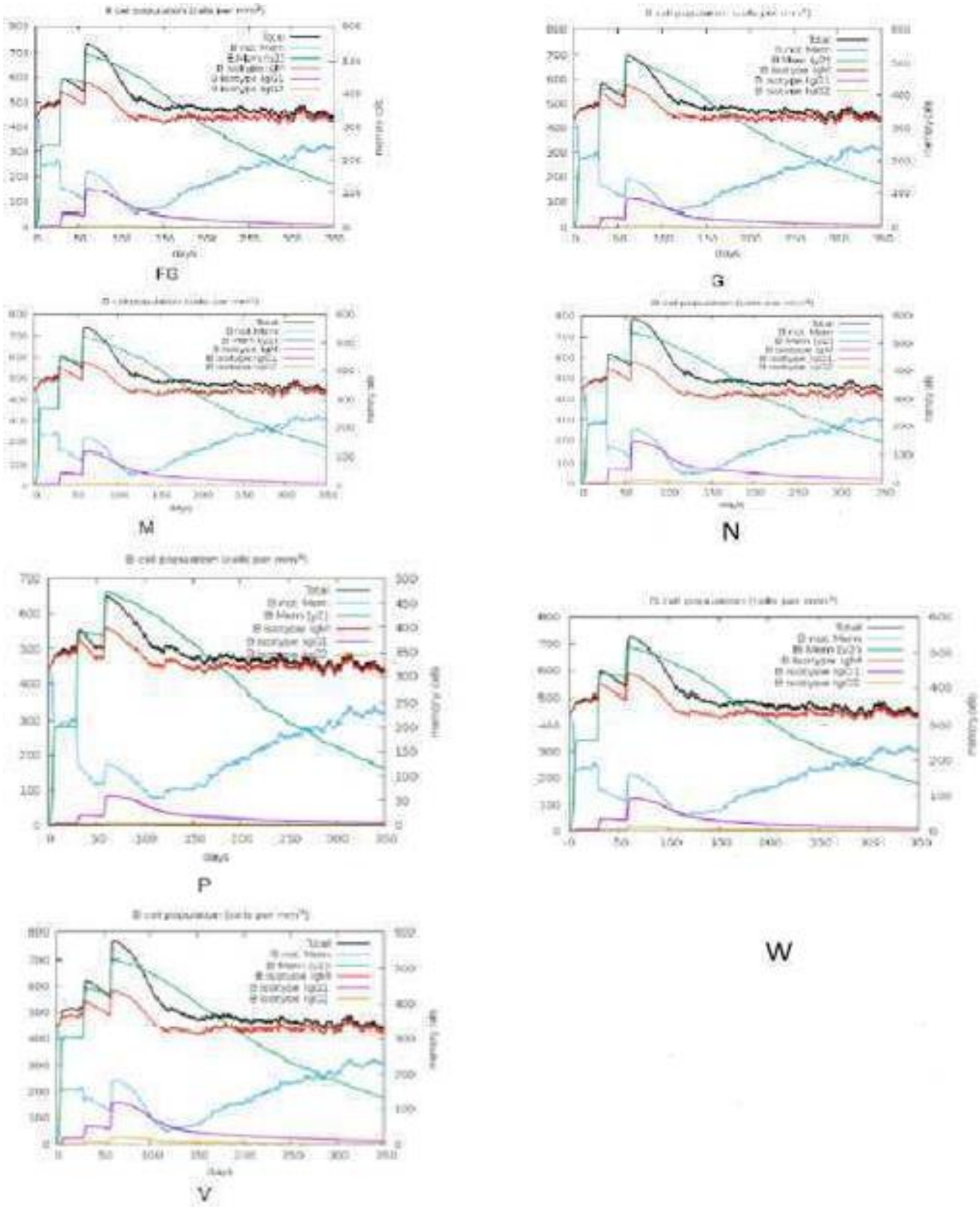


Figure 5. lymphocytes showing total count, memory cells (isotypes IgM, IgG1, and IgG2) for constructs. And plot shows total and memory counts of CD4 T-helper lymphocytes for constructs.

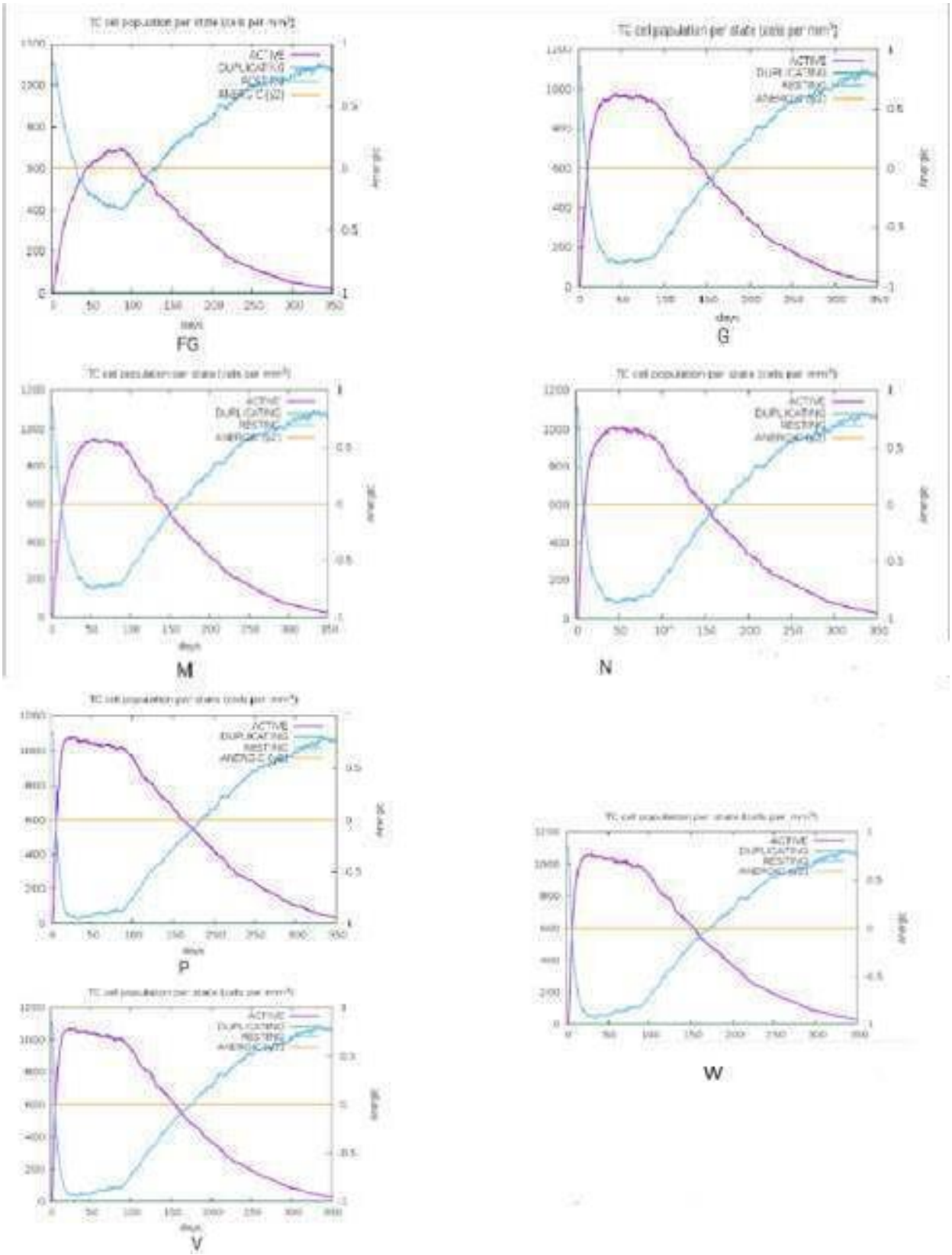


Figure 6. TC population Per stage for all constructed

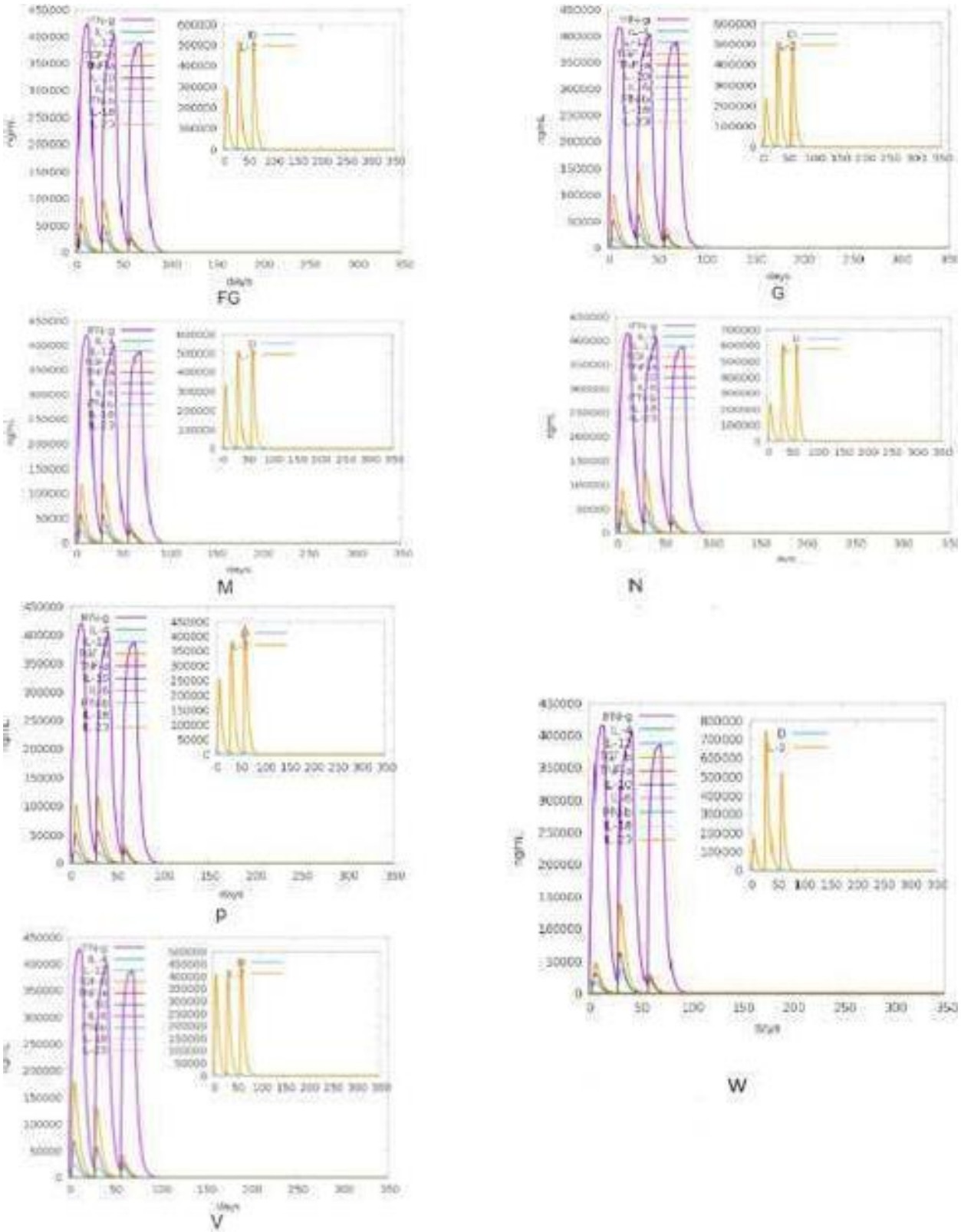


Figure 7. The concentration of Cytokines and interleukins of all constructs.

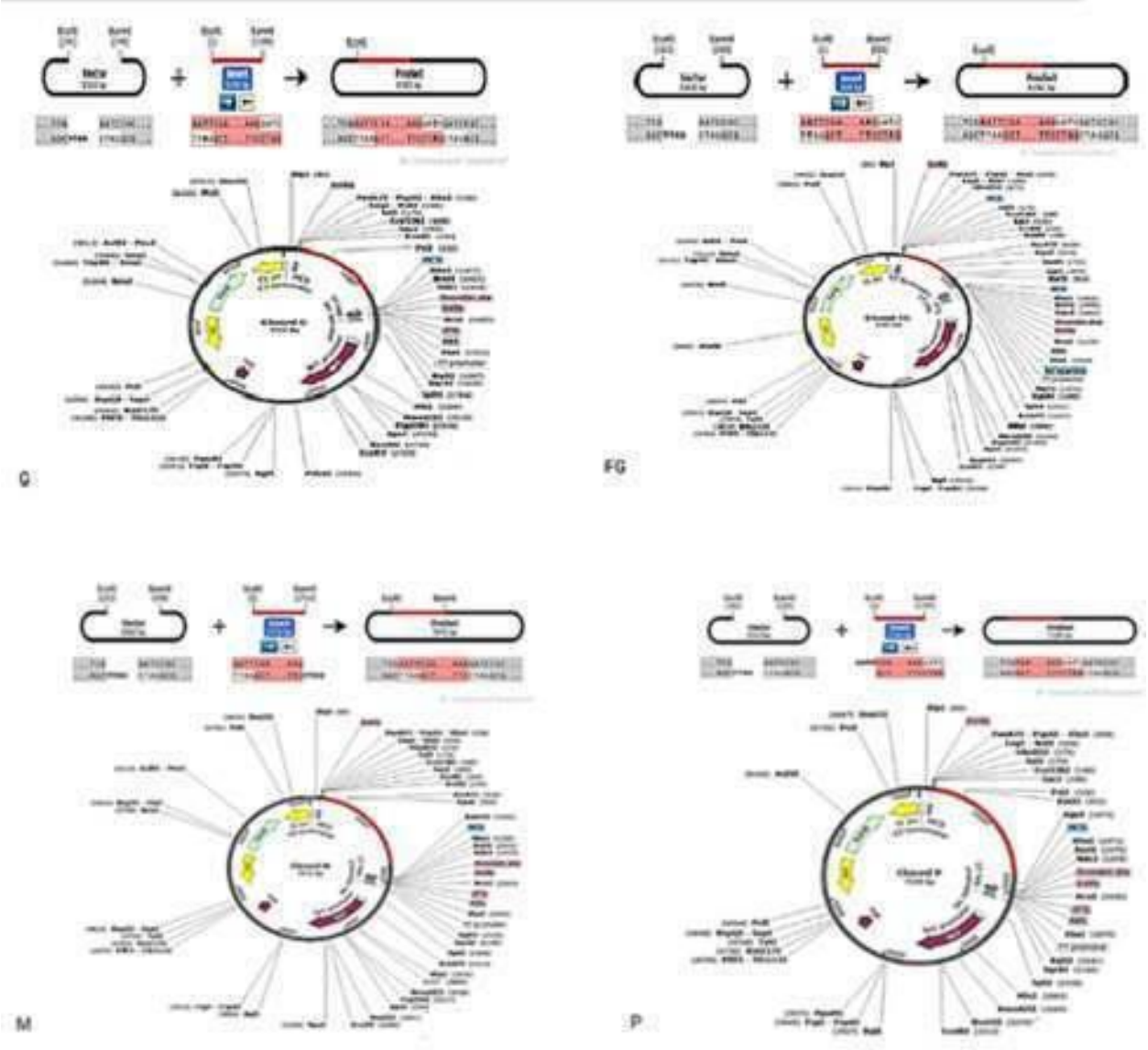
Codon Adaptation, In Silico Cloning, Prediction of the mRNA Secondary Structure, and Expression of the best Vaccine Protein with SUMO-fusion

The codon adaptation experiment of chosen eight vaccine construct (G, FG, M, P, N, L, W

and V) found out that the adapted sequence had Codon Adaptation Index (CAI) and GC content shown in (Table 12). The newly adapted DNA sequence inserted into EcoRI and BamHI restriction sites of the pETite vector plasmid. Upon translation in the E. coli host, the vaccine protein was expressed in fusion with SUMO protein and 6X His tag, which should assist effective purification and solubilization of the protein (35). (Figure8).

Table 12. Codon adaptation index and GC-content for vaccine constructed.

Vaccine Name	CAI	GC-content
G	0.98	54.2%
FG	0.92	52.1%
M	0.98	52.1%
P	0.93	54.2%
N	0.98	57.8%
L	--	--
V	0.97	51.9%
W	0.98	50.1%



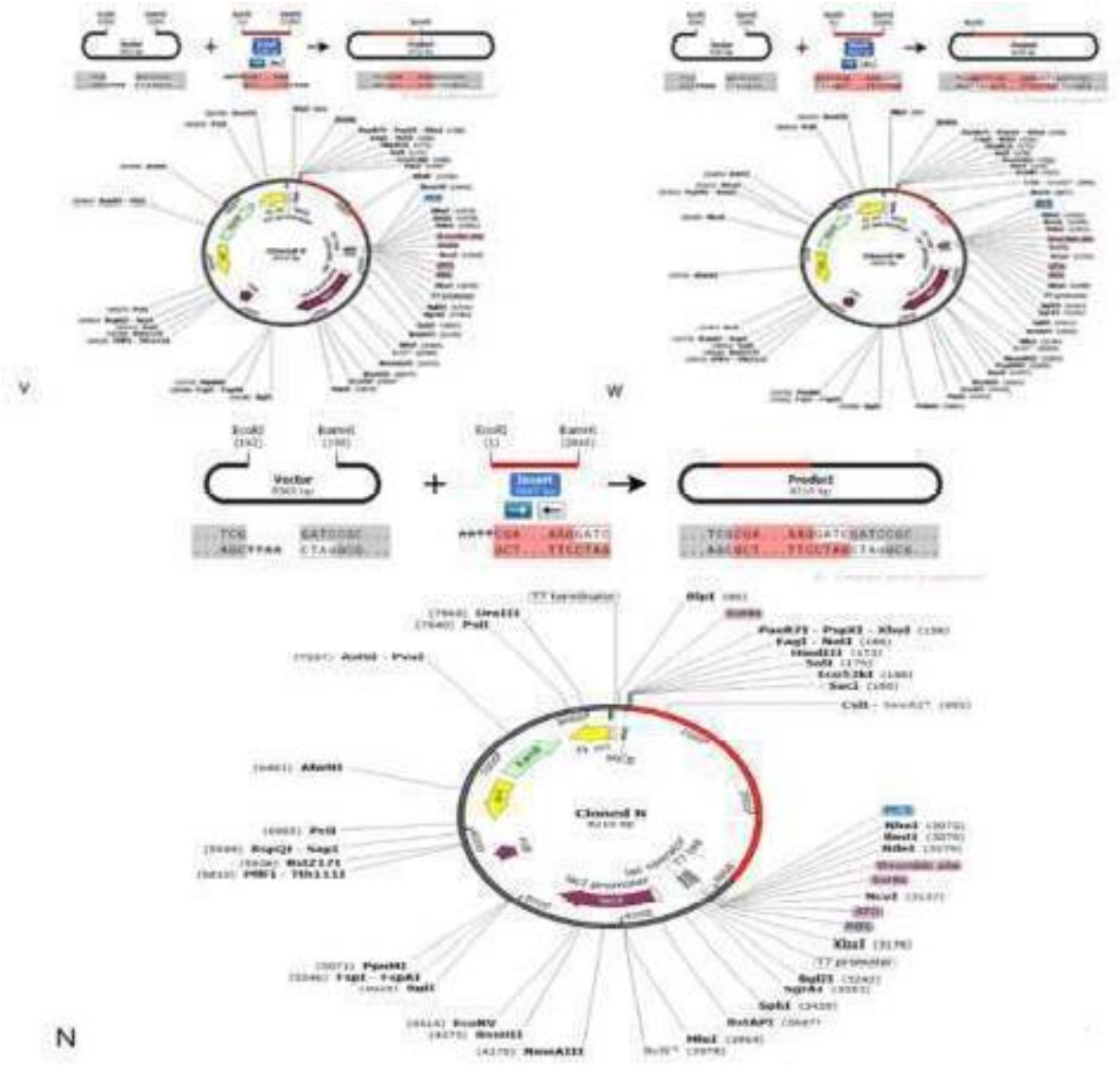


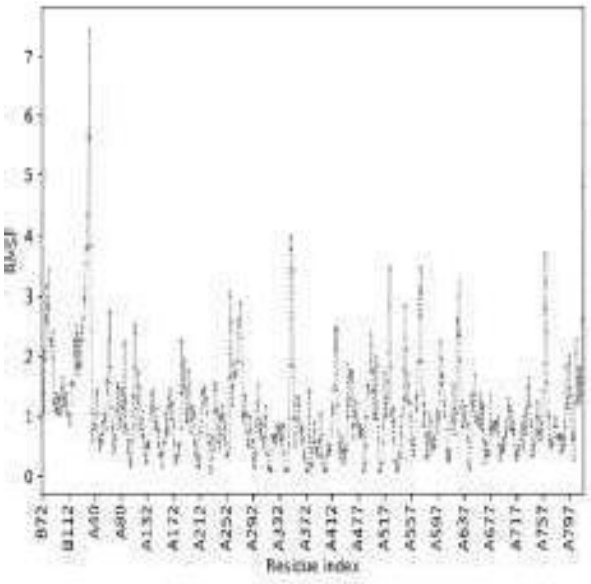
Figure 8. Expression of the final vaccine gene (Red) in a restriction cloning vector pET28a in E. coli host.

Discussion

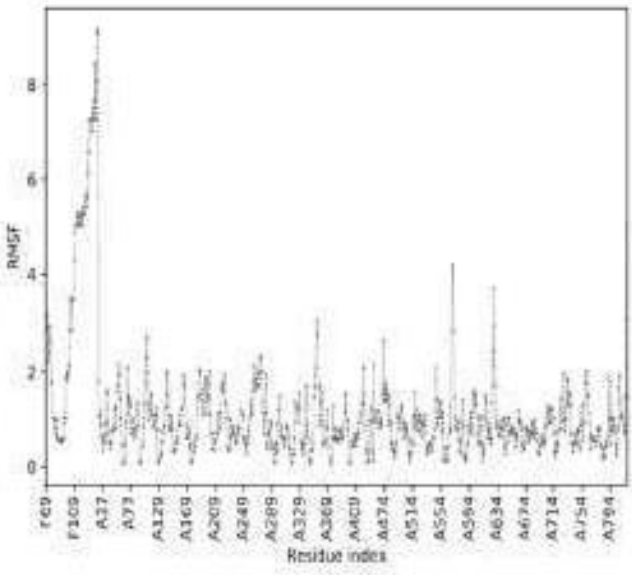
Nipah Virus is a lethal zoonotic virus with a mortality rate of 72% and 86% in Bangladesh and India respectively. There aren't yet any currently isn't an authorized Vaccine against NiV. The purpose of this study was to design In-silco vaccine against NiV proteins by using different Immunoinformatics tools. In this study, only peptides having IC50 values < 100 for MHCI and IC50<500 for MHCII have been taken into consideration as powerful peptides. All the chosen epitopes have been merged with one-of-a-kind adjuvants, linkers, and Pan-DR sequence epitopes (PADRE). PADRE sequence is responsible for the reduction in polymorphism in HLADR molecules within-side the population (36). The G-rich linker GGGS which complements the immunogenicity of the vaccine within the

host was utilized (37) a total of eight vaccine constructs (G, FG, M, P, N, L, W, V) were made. Afterwards, all the vaccine constructs were analyzed for antigenicity, solubility, toxicity and allergenicity. Additionally, the physicochemical properties of vaccines were predicted the molecular weight of the candidate vaccine G, FG, M, P, N, L, W, made it possible to predict its solubility during expression by SolPro and trigger an immune response. The theoretical pI FG and W indicating that the proteins are acidic, and G, M, P, N, L and V are basic. In addition, the anticipated instability index suggests that the vaccine peptide might be stable upon expression, as a result firming its capability for use. The aliphatic index result showed that, the vaccine can be hydrophobicity. All the above properties support constructed vaccines as promiscuous against Nipah Virus. The 3D model of the construct became constructed with the online server and validated with the aid of using Ramachandran plot analysis. The vaccines must have a great binding affinity with the MHC protein segments encoded through different MHC alleles. This is vital due to the fact that the MHC molecules produce capability immune responses after recognizing the vaccines which mimic the original viral infections. Thus, vaccines assist in producing immunity closer to a specific virus or viruses (38). TLR-8 is responsible for producing immune responses towards RNA viruses (39), (40). TLR8-specific agonists activate myeloid DCs, monocytes, and monocyte-derived DC, leading to the production of proinflammatory cytokines and chemokines, along with TNF-α, IL-12, and MIP-1α (41). The docking results showed good global energy scores for TLR8 that was used during the analysis for the vaccines, which was the indication of eliciting an optimal immune response against Nipah Virus. While the docked complex was being simulated the usage of CABS-flex (2) dynamics was enhanced it was discovered that each molecule remained stable with minimal fluctuations RMSF. The flexibility of each molecule may be visible within-side the trajectory. Simulation by C-ImmSim through a virtual injection was done for all vaccine constructed to test the proficiency of construct to the adaptive immune system. The output of the simulator became a graphical illustration of the entire count of lymphocytes, division amid isotypes, antibody, and cytokine concentration. Cells may also bind or move and follow environmental harmonized behavior (42), (43). The antibodies titers (IgM and IgG1 + IgG2) confirmed a high peak after booster doses of vaccines injection. Greater IgM production is needed for an enhanced primary immune response,

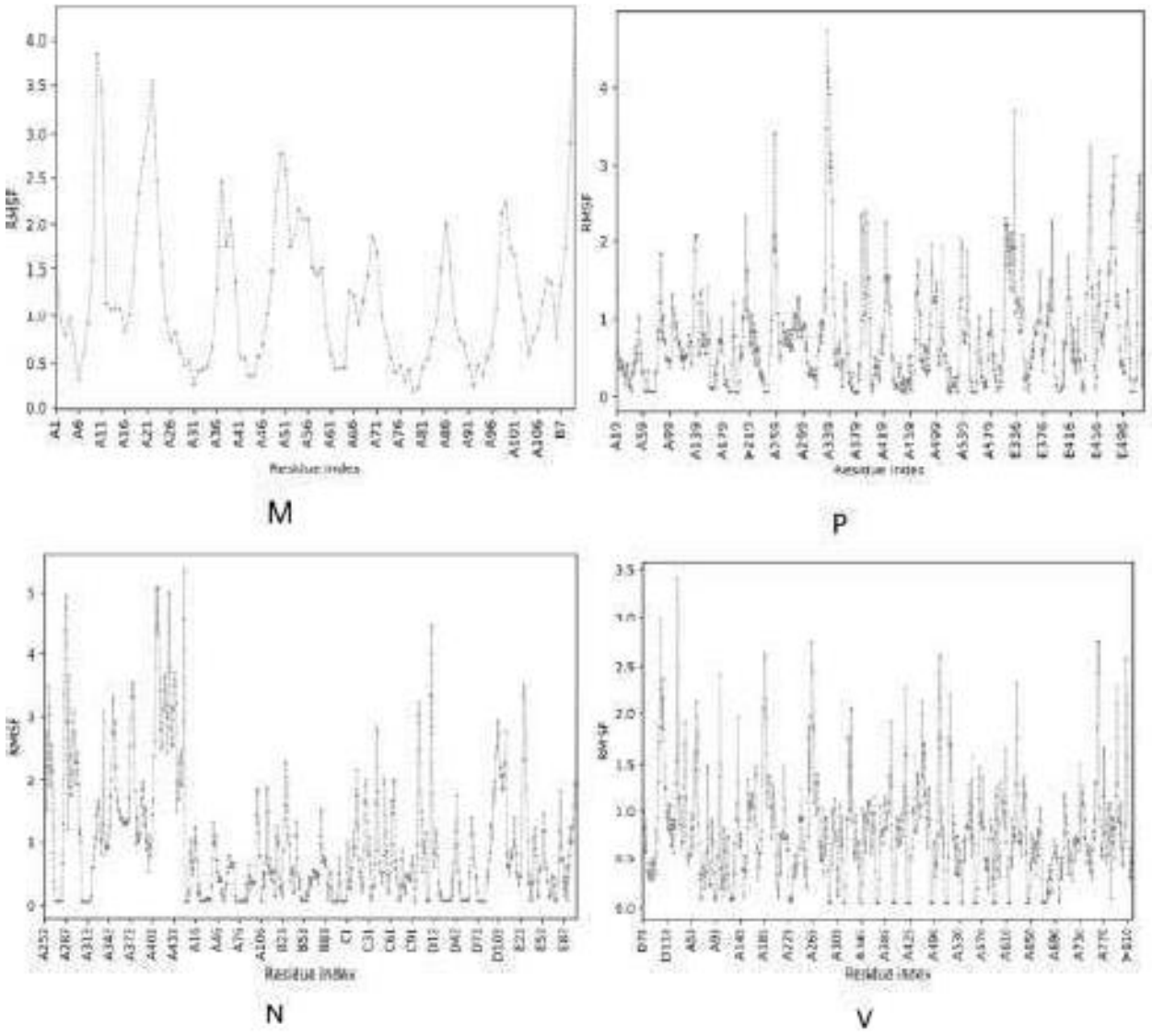
ensuing in augmentation of B cell population and further antibodies responsible for secondary and tertiary immune reactions. Overall B cell population reached its maximum across the 50th day (in cells per mm3) of injecting C1, before plateauing off. Increased CD4 T-helper cell population has an important function in evoking protection and became evoked after injection. Since the vaccines construct contained multiple numbers of B and T-cell epitopes, so it would be able to generate a diverse immune response. As a result, it can be declared that the vaccine G, FG, M, P, N, L, V and W might be able to generate a good immune response in the body. Using Snapgene, vaccine constructs turned into a cloned in silico with the assistance of the most appropriate plasmid vector pET28a (+) via the means of restriction enzymes EcoRI and BamHI to test its expression and purification in the bacterial cellular environment (Figure 8). Analysis of the virtual cloning, after codon optimization, validated the stance that translated chimeric vaccine construct seems proficient with improved gene expression and is able to vaccine production at an economical cost.

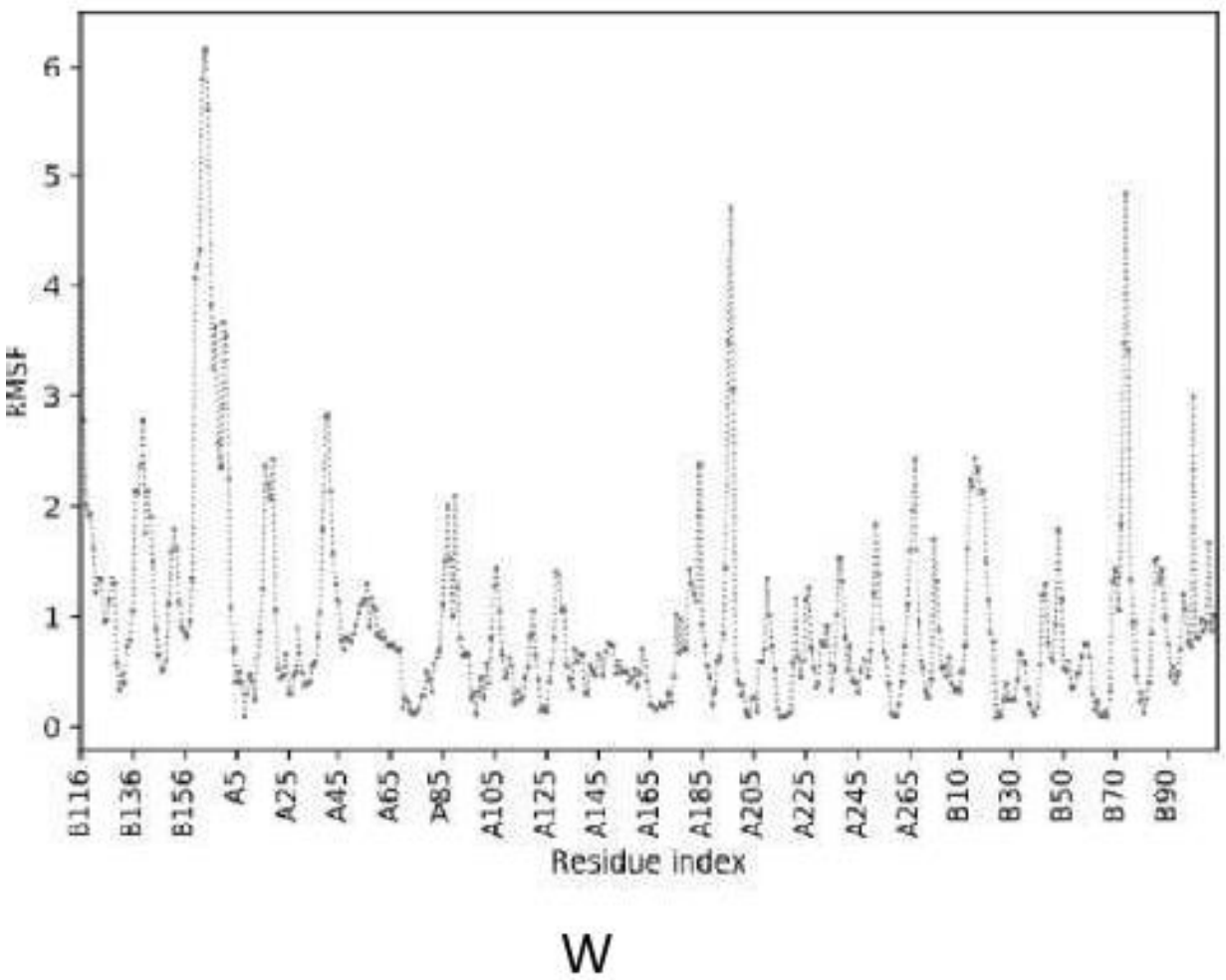


G



FG


RMSF
Residue Index
M
P
N
V



W

Figure 9. Root Mean Square Fluctuation (RMSF) plot of the protein complex during the simulation.

Conclusion

To control the spread of Nipah virus many aspects of public health must be strengthened. These include diagnostics (diagnosis), treatment and prevention. Prophylactic measures such as vaccines and mathematical outbreak prediction can reduce the mortality rates associated with viral spread. The vaccine candidate successfully stimulated both humoral and cell mediated response in immune-simulation. B-cell, T-cell, and IFN-inducing epitopes to generate a peptide-based multi-epitope vaccine which elicits humoral and cell-mediated immunity, respectively, to eradicate viral particles. Antigenicity, allergenicity, and solubility, in addition to physiochemical properties and tertiary structural analysis, had been confirmed. Predicted epitopes had been merged the use of suitable linkers and adjuvants to enhance the immunogenicity of the vaccine. After molecular docking, the selection of TLR8 depends on the best global energy against other ligands in all the Softwares that were used. MD simulation was evaluated for TLR8 and vaccines, allowing the assessment of the

binding affinity and stability of the complex. This study showed a good result for all constructed Vaccines within all software tools and tests making it possible to be a de novo vaccine against Nipah Virus except **L** construct because it could not form disulfide bonds indicating that it's not stable.

Declaration

This author does not have any applicable affiliations or financial involvement with any of the organizations or entity or financial struggles with the subject or materials mentioned in the manuscript. This includes employment, consultancies, honoraria, inventory ownership or expert testimony, presents or patents acquired or pending, or royalties.

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