



A Novel Multi-Epitope Edible Vaccine Candidate for Newcastle Disease Virus: *In Silico* Approach

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Background: Newcastle disease, is one of the most important illnesses in the aviculture industry which shows a constant threat. In this case, the vaccine could be considered an important solution to prevent and control this disease. So, the development of a new and more effective vaccine against Newcastle disease is an urgent need. Immune informatics is an important field that provides insight into the experimental procedure and could facilitate the analysis of large amounts of immunological data generated by experimental research and help to design a new vaccine candidate.

Objectives: This study is aimed at bioinformatics to investigate and select the most immunogenic and conserved epitopes derived from F and HN glycoproteins, which play a key role in pathogenesis and immunity. This strategy could cover a wide range of Newcastle disease viruses.

Materials and Method: For expression in both *E. coli* (as an injectable recombinant vaccine candidate) and maize plant (as an edible vaccine candidate) host, two constructs were designed and analyzed separately. Furthermore, the role of LTB as an effective bio-adjuvant for general eliciting of the immune system and simultaneous expressions with those two antigens was evaluated. Hence, here a multimeric recombinant protein with the abbreviation LHN2F from the highly immunogenic part of HN, F and LTB proteins were designed. The synthetic construct was analyzed based on different bioinformatics tools.

Results: The proper immunogenicity and stability of this multimeric fusion protein have been shown by immunoinformatic methods from various servers. To confirm the function of the designed protein, the final molecule was docked to chicken MHC class I using the Pyrex-python 0.8 program. the results of Immune Epitope analysis were confirmed by the docking results between protein and receptor.

Conclusions: The results of structural and immunological computational studies proposed that the protein deduced from this novel construct could act as a vaccine candidate for Newcastle disease virus control and prophylactic.

Keywords: HN and F glycoproteins, Immune informatics, LTB bio-adjuvant, Vaccine Candidate, Newcastle disease virus,

1. Background

Newcastle disease (ND) is a crucial epizootic disease that attacks the avian respiratory. These can cause an enormous economic impact on the global scale (1). According to Office International des Epizooties (OIE), ND is a notifiable disease that creates economic barriers to trade in poultry products among countries (2). Newcastle

disease virus (NDV) infects many species of all avian domestic and wild populations in most countries (3). The NDV particle contains a non-segmented single-stranded and negative RNA genome and six genes that encode eight proteins (4).

The viral infection started with the interaction of two important proteins, the fusion protein (F) and the

hemagglutinin-neuraminidase (HN). HN detects and binds sialic acid receptors on the host cell surface to promote fusion through interacting with F protein. Besides, HN possesses the neuraminidase activity to hydrolyze sialic acid from progeny's virion particles to prevent virus self-accumulation. F protein mediates the penetration of the virus content into the host cell and the fusion allowing the viral RNA enters to the cytoplasm. These two surface proteins (HN and F) are important targets for the host's immune response and antibodies against the F or HN can neutralization the virus (4-6). Due to the fast dissemination of ND, probably caused by the intense commercial exchange in the poultry industry and the constant threat of the virus from wild birds, the safety in poultry establishments and the need for preventive actions have become imperative. This disease could be controlled in many different ways, which production and application of vaccines can be promising through all those failures (1, 2). But regardless of available vaccines, this epidemic is still among increasing threats. It means revolving into a new vaccine should be scheduled by researchers (1, 3, 7). After introducing the concept of reverse vaccinology, the new vaccine candidates are selected based on *in silico* tools. In this new vaccine design strategy, the conserved and effective epitopes, which have the smallest part of the antigen's protective activity, instead of using the whole antigen, were used. The advantages of this approach include reducing production costs and a more detailed design of the subunit vaccine. Recombinant subunit vaccine technology could open a new and promising horizon to conquer many epidemics (8-11). It has been shown that the heat-labile enterotoxin B subunit (LTB), derived from toxigenic *E. coli* or B subunit of *Vibrio cholerae* are powerful mucosal and parenteral bio-adjuvants. Moreover, they effectively incite mucosal and systemic immune responses to the robust immune response against antigens fused to these molecules (12-15). Several antigens fused to LTB have been reported to induce immunity in poultry experiments (14, 16-18).

2. Objective

In this study, we focused on the Immune informatics design of a recombinant edible subunit vaccine candidate to stimulate the immune system in poultry, through elicit of neutralizing antibodies against HN and F glycoproteins, NDV can neither adhere to the

surface of the mucosa nor enter into the cell. The study aims to design the short immunogenic and conserved epitopes derived from F and HN proteins, with no demand for larger antigens, against a wide range of NDV, via immunoinformatic tools. Moreover, the HN was doubled (HN-HN) to enhance HN immunogenicity and utilize immunogenic and conserved epitopes containing a known bio-adjuvant (LTB) for improving the immunogenicity of the designed vaccine. Some appropriate linkers were also employed for more optimal protein folding and interaction with the immune system. All the sequences of the fragments are provided in a single-gene cassette for simultaneous expression. However, it is needed that the bioinformatic assessment confirms its efficacy and show that this designed construct can be applied as a potential candidate vaccine against NDV.

3. Materials and Methods

3.1. The Sequence of *ltb*, *hn* and *f* genes and Epitopes Prediction

The complete amino acid sequence of the LtB (P0CK94), F (P33614) and HN (P35743) were retrieved from UniProt database in the FASTA format. The linear and conformational B-cell and T cell epitopes were analyzed based on crystal structure and Bce pred, Discotop, IEBD, CB Top, Elii pro, BC pred, Pro pred-1, ABC pred, Bepi pred, NetMHC4 and MHC 2 pred web-based servers (19-24).

3.2. Conceptual Construction of a Multi-Epitope Vaccine

Due to the minor immunological effects of the hemagglutinin structure (5, 14) and to increase the immunogenic properties of the *hn* gene, its sequence in the chimeric structure was duplicated (increase the antigenic dose). The arrange of the selected epitopic area of *ltb*, *hn* and *f* were attached with the proper repetitions of appropriate peptide linkers (EAAAK) (25). The synthetic gene structures were evaluated by the Modeller 9.18 program in different arrangements. The 3D structure was examined, and antigenicity (Vaxijen, SCRATCH and EMBOSS) and allergenicity (Alg pred, SDAP, ADFS, Aller top, Allergome) were estimated for all models. Finally, the best model (the LTB-HN-HN-F arrangement) based on the maximum value of the structure stability, antigenicity and minimum value

of the allergenicity was selected. The initial amino acid Met, for transcriptional initiation, was added at the beginning of the construct. The 6xHisTag sequence and the SEKDEL (26) retention signal was added to the C-terminal of the final construct to facilitate the purification and accumulation of the final protein in the endoplasmic reticulum, respectively (9, 11, 15, 22-24).

3.3. Prediction of Protein Structure and Molecular Dynamic

The secondary structures of LHN2F were predicted by different online servers (GorIV, PSI pred, PHYRE, Porter, SOPMA and SCRATCH). 3D structure and homology modelling for the final multi-epitope peptide was performed by I-TASSER, RaptorX, Swiss-model, Modfold6 and Modeller 9.18 servers.

The PolyView-2D protein structure visualization server was used to generate and visualize schematic annotations of secondary structure, and the DS visualizer 1.7 was applied to visualize the modelled 3D structures (11, 21, 27). All of the 3D models were estimated using Protein Structure Validation Software (PSvS), ProSA-web and SuperPose server for validation of the structural quality and comparison with native structures to identify possible errors in modelled structures. A validation quality process was performed to identify potential errors in the 3D structures, identify better fits of the model in predicted structures and comparing the model before and after the refinement by use score and root mean square deviation (RMSD) (15, 23, 28). Finally, molecular dynamics simulation prediction was applied to determine the best-designed model's dynamic stability by GROMACS package. In this method, the interaction between atoms and molecules over time is simulated by computer-based physics laws. The main outputs of this analysis are RMSD and RMS Fluctuation. The RMSD index is used to evaluate the simulated structure's stability (21).

3.4. The Physicochemical Analysis of Chimeric Protein

Various physicochemical properties were assessed and performed using the ExPassy protparam online database. Besides, the chemical characteristics and Ramachandran analysis were examined (11, 29). The multi-epitope fusion protein's solubility was evaluated by proso 2 and scratch server. The accessibility of solvent was assessed by pdsv software (11).

3.5. Molecular Docking Studies

To study the peptide-protein interactions between designed protein and chicken immune receptor (MHC), the molecular docking as convenient technique were applied. Based on NetMhc4 prediction tool (<http://www.cbs.dtu.dk/services/NetMHC/>) to analysis of peptides binding to MHC class I molecules, these epitopes were docked with chicken MHC class I haplotype B21 (BF2*2101) receptor (30-34). The crystal structure of BF2*2101 was retrieved from UniProt database with PDB ID of 3BEW. PyRx python prescription 0.8 tool (<http://pyrx.scripps.edu/>) was performed in this study. Visualization was carried out using UCSF-Chimera tools.

3.6. Immunological Analysis

To ensure that the epitopic area in the final structure of the LHN2F is exposed, the exposure and accessibility of the epitopes were re-examined using the algorithm of linear and conformational B-cell and T cell epitopes servers. Also, the interferon-gamma inducing epitopes are predicted by the IFN epitope server for stimulating cellular immunity (19-23, 35).

3.7. Localization, Codon Optimization and mRNA Stability

Psl pred, PSORT and WOLF software were used to investigate the target protein cellular localization in *E.coli* and maize expression hosts (24). The synthetic construct was codon optimization for expression in the desired prokaryote (*E. coli*) and eukaryote (maize plant) host by different online software (IDT DNA, optimizer, cool, Jcat, and Genscript). The presence of unstable factors, methylation area (DAM/DCM) and restriction enzymes limited effects on DNA levels were investigated and deleted (11). The secondary structure of optimized mRNA from the *lhn2f* gene was predicted by Mfold software in terms of the minimum energy value. The minimum energy of the predicted mRNA structure was compared with the pre-optimization state (36). Finally, appropriate restriction sites were introduced to the 5' and 3' ends of the sequences. Two recombinant genes were synthesized by Biomatics Company (Canada), and the synthetic construct designed was registered in the NCBI database with access No. MH023426. All the immunoinformatic steps required for reverse vaccinology are shown schematically in **Figure 1**.

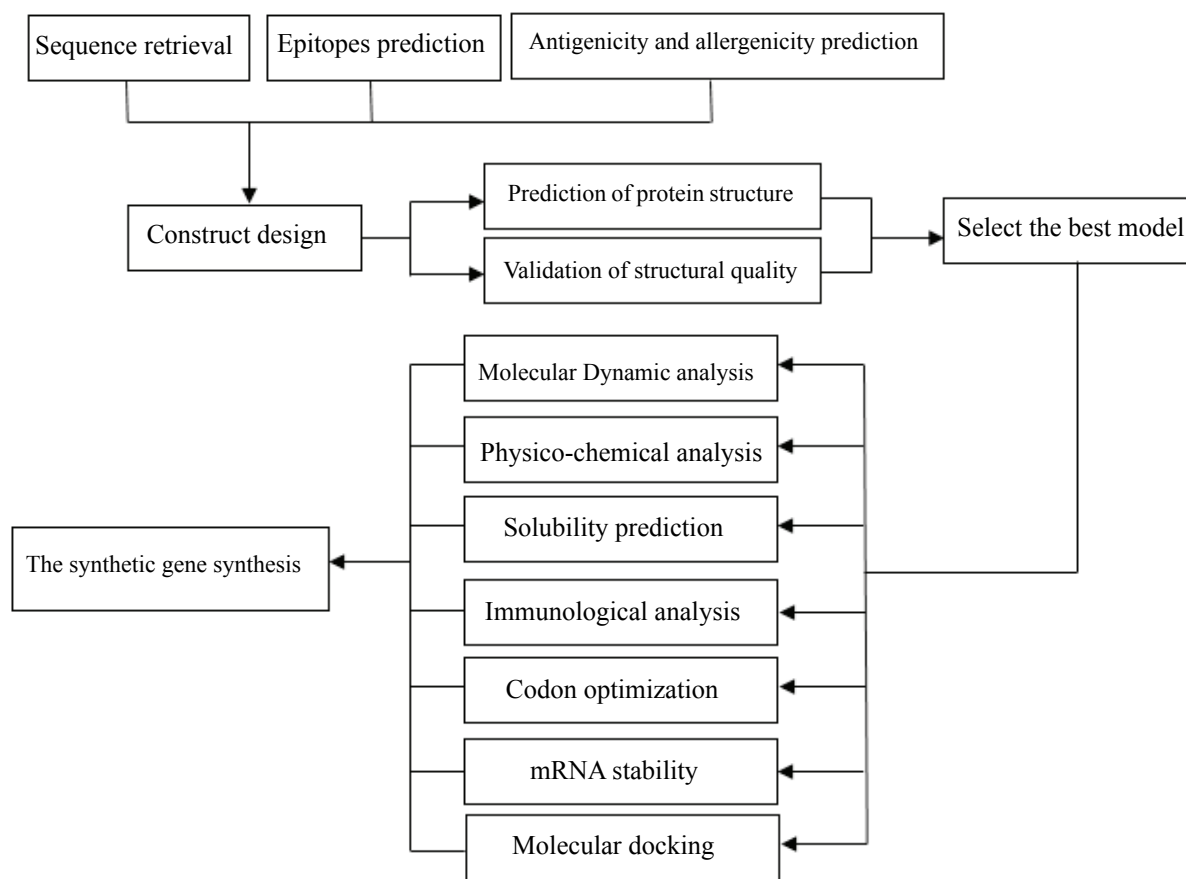


Figure 1 . Schematic representation of the workflow to design a multi epitopic vaccine candidate.

4. Results

4.1. Construct Design and Analyses

The high-scored epitopes were selected and incorporated in the final vaccine candidate construct. The linear and conformational B cells and T cells epitopes were analyzed in the LTB-HN-HN-F order as the best model, and repeats of EAAAK sequences separated each domain. The BLAST search of the final sequence revealed up to 98% homology and similarity between LaSota (RefSeq) and VII (Iran) VD virus strains. The 6×His-tag for affinity purification and the SEKDEL retention signal sequence (just for eukaryotic protein) were added to the C-terminal of sequences. The sequence designed with 458 amino acids was further evaluated. The schematic representation of the final construct showed in **Figure 2A**.

4.2. Prediction of Antigenicity and Allergenicity

The antigenicity of recombinant protein was predicted

0.928353%, 0.4508% and 21% by ANTIGENpro, Vaxijen and EMBOSS. The results showed that the fusion and components' antigenicity with the highest antigenicity index probability was optimal and has been above the threshold defined by the software. It confirms that the selected areas are antigenic and have good antigenicity. The allergen analysis of the fragment also proved that the fusion of the designed protein is not classified as an allergen protein.

4.3. Prediction of Secondary and Tertiary Structures of the Chimeric Protein and Molecular Dynamic

The chimeric protein's secondary and tertiary structure was predicted, and the best model is illustrated in **Figure 2B** and **C**. All results aligned by native structure and the second structure generated by GORIV servers have the most fitted structure to the native proteins (data not shown). According to these data, the structure was consisting of near 40% alpha-helix (H), 22% extended strand (E) and 38% random coil (C). The value of the 3D

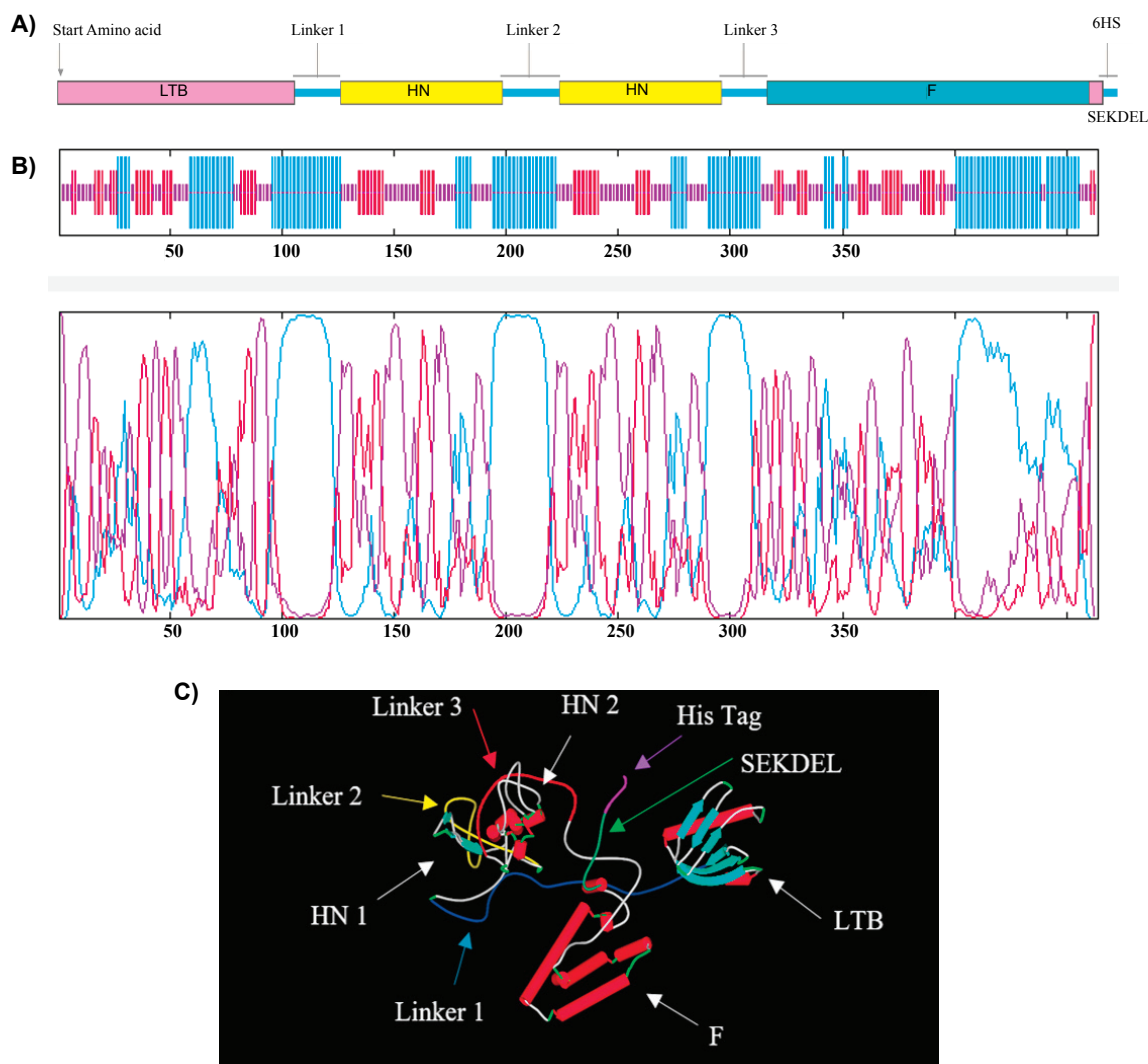


Figure 2. A) Schematic representation of LHN2F fusion protein with linkers and other added features. Start and stop codon was added to the beginning and end of the sequence. B) Prediction of the secondary structure of the LHN2F chimeric protein by the GORIV. C) Three-dimensional structure of LHN2F protein by PDSV software.

structures modelled was evaluated by the PSvS server (PROCHECK, RAMPAGE and MolProbity). The best result is the model predicted by Modeler with a global model quality score of 0.2136, and I-TASSER has the least score (the score is between 0 and 1 and scores less than 0.2 is significant). SuperPose performed the authenticity of the model to estimate the quality of the predicted model. The model predicted by Modeler is more comparable to the native structure than the other models, and I-TASSER has the lowest score (data not shown). In depicted plots for LHN2F structure, the calculated Z-scores (-2.81) is within the range of scores typically found for native proteins. The Swiss-

Pdbviewer energy minimization calculation shows that the predicted Modeler and Mold fold models for LHN2F (-10305.188 $\text{kJ}\cdot\text{mol}^{-1}$) are more stable than the other models. The calculated normalized B-factor for LHN2F is classified in the range of stable proteins (**Fig. 1, Supplementary data**).

MD analysis was performed to evaluate protein stability and for this purpose the GROMACS software was used at the simulation length of 100 ns. The RMSD chart should reach a plateau and be stable after a while. The protein in this chart reached stability after approximately 20 nanoseconds which indicating the protein stability. Another diagram, the RMS Fluctuation shows the

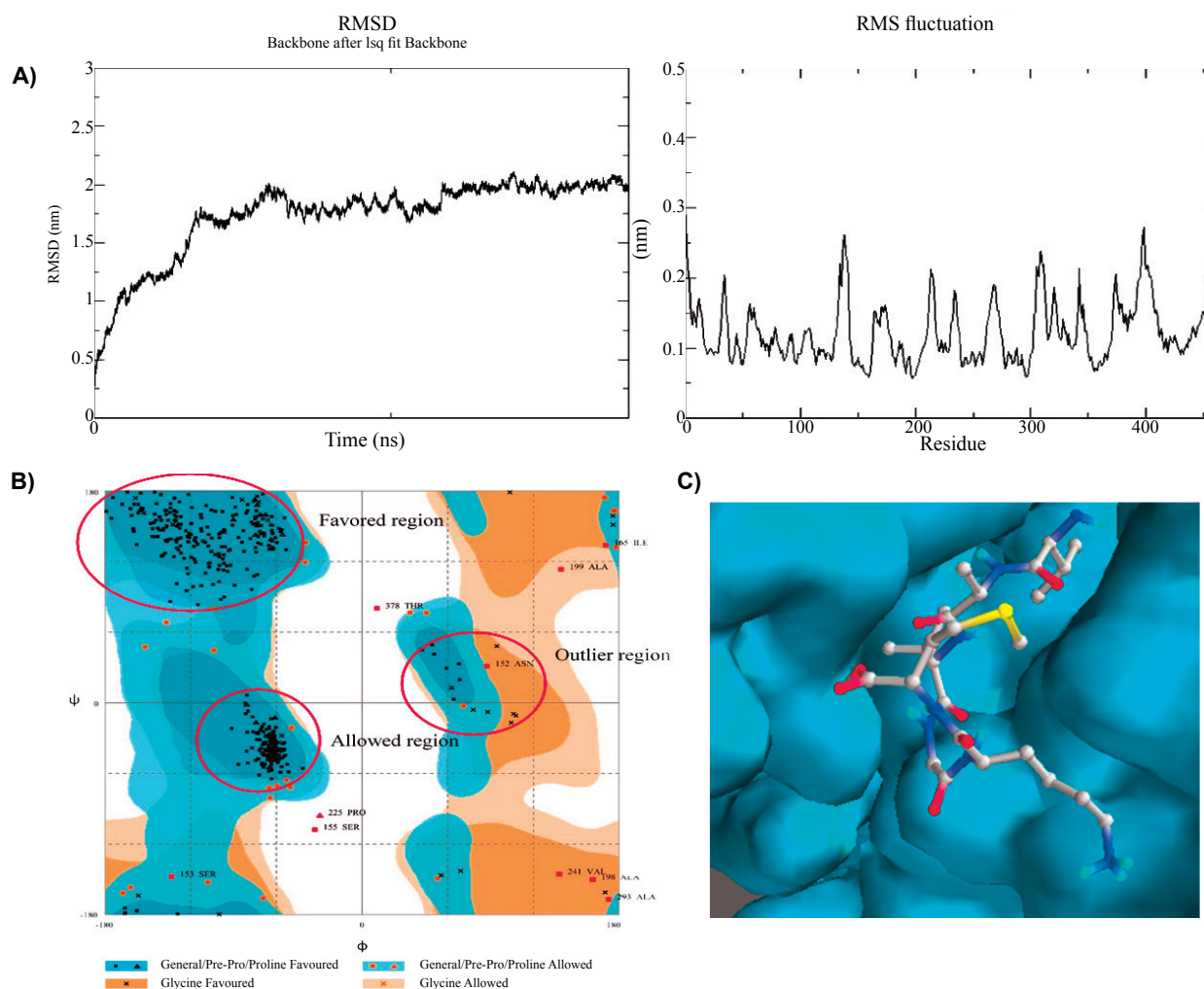


Figure 3. A) Molecular dynamics for LHN2F protein. In the RMS diagram, residues above zero are valuable and the normal range is 0.2. For detail see the text. B) Evaluation of the quality of the model designed based on the Ramachandran diagram. C) Interaction between the most prevalent epitope (DTCPDEQDYQ) and receptor (BF2*2101 allele) after docking by the PyRx program.

degree of fluctuation of different parts of the protein which no fluctuations outside the allowable region were observed in the protein (**Fig. 3. A**). Finally, it was observed that the output protein PDB of MD, which indicates the structure of the protein after stimulation, was not different from the structure before analysis.

4.4. Physico-Chemical and Stereochemistry Analysis

Localization analyses predicted this recombinant protein to be localized and targeted either in the cytoplasm and According to the PROSO II server analyses, the designed recombinant protein was classified as a soluble protein (Solubility score 0.767, the threshold:

0.6). Molecular weight (MW), isoelectric point (pI) and the high aliphatic index of the fusion protein were computed 49.7 kDa, 8.84 and 77.35, respectively. The estimated protein half-life was more than 10 hours in *E. coli*, *in vivo*. The protein instability index was 35.70, which indicates that this protein is classified in the group as stable proteins (40 value and more is predicted to be unstable). Also, the Grand average of hydropathicity (GRAVY) was -0/378 so our protein was classified as a hydrophilic group. The thermal stability of globular proteins (high aliphatic index) and the low GRAVY score, indicates the better interaction between LHN2F protein and water. Ramachandran plot statistics

Table 1. The docking results. binding affinity for proposed MHC I epitopes to BF2*21:01. the lower energy needed for binding is the better.

Epitope (ligand)	binding energy (kcal/mol)	No. Cluster
DTCPDEQDYQ	-8.4	8
SIAATNEAV	-7.9	9
SQLAVAVGKM	-7.4	8

Table 2. Prediction of linear B-cell epitope regions of HN and F glycoproteins in LHN2F protein fusion at Bcepred site.

Prediction parameters	Threshold	Epitope
Hydrophilicity	1.9	11-17, 79-85, 90-96, 103-120, 150-162, 167-180, 195-216, 246-258, 263-276, 291-307, 315-321, 322-330, 340-348, 373-386, 414-420
Flexibility	2	57-63, 87-94, 174-155, 182-188, 243-252, 278-284, 321-328, 363-369, 374-384, 447-454
Accessibility	1.9	10-20, 30-37, 57-86, 88-97, 103-120, 148-194, 200-216, 244-290, 296-307, 323-329, 336-348, 350-360, 367-376, 450-461
exposed surface	2.3	58-74, 79-86, 90-96, 150-156, 162,172, 179-182, 246-252, 258-268, 270-278, 337-346, 450-457
Polarity	1.8	8-14, 30-41, 58-74, 79-86, 103-120, 162-168, 200-216, 258-262, 296-307, 338-345, 368-376, 420-429, 431-439, 450-464
antigenic propensity	1.9	39-45, 48-59, 82-89, 139-149, 163-169, 235-245, 259-265, 329-339
Terns	2.4	150-157, 246-252, 456-464

show that the Modeler model has more amino acids in the preferred region than the other 3D models (data not shown). According to the Ramachandran diagram, 92.6% of amino acids are in the favored region, 5.2% are in the allowed areas and only 2.2% of amino acids are in outlier areas, which confirms the correct design and folding of the protein (**Fig. 3B**).

4.5. Molecular Docking Analysis

All proposed epitopes docked to BF2*2101 allele. Results showed that all peptides have good scores and affinity (data not show). The lower bonding affinities are shown in **Table 1** and **Figure 3C**. The best epitopes including ¹⁷⁰DTCPDEQDYQ¹⁷⁹, ²⁶⁶DTCPDEQDYQ²⁷⁵ (due to repeat of HN proteins), ⁴²⁷SIAATNEAV⁴³⁵ and ⁴⁴³SQLAVAVGKM⁴⁵² had encouraging results.

4.6. Prediction of B, T Cell and Interferon-Gamma Inducing Epitopes in LHN2F

The epitopes were examined to ensure the exposure of the epitopic areas of LHN2F. The results confirm the presence and exposure of the epitopic sites. Only the high-scored epitopes are listed in **Tables 2 and 3**.

4.7. Analysis of The Gene Construct

Two codon-optimized sequences by Genscript were reverse translated into the amino acid sequence by Translate tool–ExpASy (<https://web.expasy.org/translate>) and the result showed the unchanged amino sequence. Codon optimization increases CAI to 0.91 for prokaryotic and to 0.92 in the eukaryotic gene. Also, the GC content was optimized to 56.23 and 64.44 in the prokaryotic and eukaryotic genes, respectively (**Fig.2, Supplementary data**). The minimum energy of the predicted bacterial and plant mRNA structure shows the structural stability of the optimized gene. In both mRNA structures, the essential cis-elements (ATG, ribosome binding site (RBS) or Kozak sequence)

Table 3. Prediction of discontinuous B-cell epitope regions of LHN2F protein at Ellipro site (Threshold: 0.5), IFN-gamma inducing epitopes by IFNepitope server and MHC class-II epitope regions at MHC 2pred site (Threshold: 0.5).

	Epitope	Score
discontinuous B-cell	MAPQSITELCSEYRNTQIYT	0.847
	ESVTTSGGGRQGRLLIGAIIGGVALGVATPAQITAAAALI	0.847
	ATFQVEVPGSQHIDSQKKAIERMKDTRLRI	0.799
	YTESMAGKREMI	0.75
	PNLPKDKEACAKAPLDAYNR	0.721
	DKLCVWNNKTPNSIAAISMEN	0.678
	VANYPGVGGGSFIDSRVWFSV	0.67
	IYKRYNDTCPDE	0.639
	GGSFIDSRVWF	0.6
	KRYNDTCPD	0.6
IFN-gamma	TPAQITAAAALIQAKQNAANILRLKESIAA	5.18
	DKAVNIYTSSQTGSIIVKLLPNLPK	3.11
	CVWNNKTPNSIAAISMEN	0.52
	NEAVHEVTD	0.36
	AVAVGKMSE	0.26
	LDAYNRTLTLTLLT	0.058
MHC class-II	LCSEYRNTQIYTINDKI	
	ERMKDTLRITYLTETKI	
	CVWNNKTPNSIAAISMEN	
	DKAVNIYTSSQTGSIIVKLLPNLPK	
	LDAYNRTLTLTLLT	
	LGDSIRRIQESVTTS	
	TPAQITAAAALIQAKQNAANILRLKESIAA	
	NEAVHEVTD	
AVAVGKMSE		

which is necessary for exact and efficient translation are located in a loop structure which could facilitate the translational procedure (**Fig. 3, Supplementary data**). The minimum energy content of the bacterial and plant mRNA structure is -5388.20 and -618.40 kcal/mole, respectively.

5. Discussion

The control of ND will not be achieved without novel control methods. According to the guidelines published by OIE, the Newcastle vaccine is graded as one of the crucial treats which emphasize the urgency of research attempts in that field. prevention vaccination is done in all but a few countries that produce chicken on a commercial scale (2). Due to the geographical

distribution reported by OIE, the disease has been controlled in some countries but continues in other areas. However, because wild birds naturally can carry the virus without getting sick, it can spread wherever the chicken is raised. Therefore, the importance of bird safety is clear.

Producing safe vaccines for conventional, reappeared or new appeared infection agents is a time-consuming and expensive process. Recent advances in immunobiology and bioinformatics science have demonstrated that researchers can design effective vaccines, particularly in infectious diseases, through computational methods. Bioinformatics approaches as a valuable tool in biological research have been used to identify candidate protein vaccine antigens which offer more rapid advances towards

preclinical vaccine studies. *In-silico*-based research provides a powerful and reliable resource for structural stability, energy quantity, and protein functionality analysis. These new abilities could save time, reduce costs, design several conceptual structures, and gather significant results with simulation steps. Besides, a candidate antigen's immunoinformatic analysis can proceed to a proper selection of immunogenic agents for a chimeric multi-subunit vaccine.

Different studies show that the glycoproteins of hemagglutinin-neuraminidase (HN) and fusion (F) play a vital role in the infection, pathogenesis and immunity of NDV. The main immunogenic part of HN and F were mainly distributed between the amino acid residue 25-550 and 50-470 respectively (5, 9, 37-41). Different *in silico*, *in vitro*, and *in vivo* studies have focused on these proteins as components of the vaccine against NDV. Still, here a novel subunit vaccine candidate composed of short epitopic protein segments along with bio-adjuvant (LTB) and a double HN structure was designed and further analyzed using immunological bioinformatics tools and reverse vaccinology. In vaccine formulation, effective adjuvants are a crucial requirement and play an essential role in improving vaccines' effectiveness. Each gene's immunological hotspots were analyzed separately to achieve the best arrangement sequence from LTB-HN-HN-F, first and the epitope prediction servers aligned the results. The highest antigenic index of all possible arrangements of the segment was examined. The 3D models generated and the hypothetical proteins were designed in all possible arrangements. The LTB-HN-HN-F array (LHN2F) was selected as the most appropriate form (**Fig. 2A**). Moreover, immune informatics analyses indicated that the fusion construct is a robust antigen and non-allergen. The EAAAK peptide repeat is used as a linker to facilitate the folding of multimeric proteins with flexibility and help to expose epitopes of domains with efficient separation of segments. The Ramachandran plot and PSvS server results showed that the quality of the model has been adequate considerably. According to the MW, instability index fusion protein high aliphatic index and low GRAVY, it can be resulting that the protein is a stable, thermostable and hydrophilic and proper antigen. MHC I prediction online tools do not support chicken MHC class molecules and several studies suggest that chicken's B-F and B-L molecules are similar and homologous to mammalian MHC class

(30-34). According to docking results, four conserved epitopes founded have the best interaction with BF2*2101. The work of Mayahi *et al* (32) and Hosseini *et al* (30) confirms these results. DTCPEQDYQ of HN protein ranked as the best based on PyRx score. So, this epitope was predicted to bind to chicken immune receptors with high affinity. This ligand has two times repetition in final fusion protein. Two other ligands related to F protein and have a good affinity with BF2*2101. Based on these results, SQLAVAVGKM were found to be target for both MHC class I and class II. All of these ligands were predicted in part of linear and conformational B-cell epitopes. Taken together, immune informatics tools including web-based server's prediction epitopes and docking, and epitope mapping by monoclonal antibodies demonstrated that the identified neutralizing epitopes need to be tested by *in vitro* and *in vivo* experiments to support new chimeric vaccine candidate for chicken.

One of the most critical steps in designing a subunit vaccine is the selection of the best B-cell (linear and discontinuous), T-cell (epitopes which have close binding affinity >1000 to proper MHC molecules), epitopes and interferon-gamma inciting epitopes for stimulating humoral and cellular immunity, respectively. These are the essential factor for the prediction of the antigenicity of any epitope-based vaccines. Identification and characterization of effective and functional epitopes could play a vital role in drug design, immunodiagnostic tests, vaccine, and antibody production. Reputable servers in this study have investigated potential epitopes of the designed vaccine candidate, and results show the synthetic protein could incite humoral and cellular responses efficiently. The molecular dynamics analysis showed this complex structure was more stable throughout the molecular dynamics simulation (100 ns). Based on RMSD analyses of LHN2F, the chimeric protein is in the normal range and does not have any structural changes during the study and reaches a steady situation at 20 ns. The RMS as an amino acid fluctuation index is in the normal range (0.2 nm).

Finally, for efficient protein expression of the chimeric protein in the *E. coli* and maize hosts, various parameters like CAI, CFD, GC content, folding and energy of mRNA of the genes were optimized. This level of mRNA energy in plants and bacteria indicates the stability of mRNA in translation. All the indices, as

mentioned above, showed that our fusion protein could be well expressed in the hosts.

6. Conclusion

In this study, fusion protein consists of epitopic part of HN and F glycoprotein of Newcastle virus with LT-B Bio-adjuvant were designed and evaluated using various immunological bioinformatics methods. The results of structural and functional predictions of the *in silico* and immunological methods showed that synthetic protein was the best form and can be used as a preventive vaccine for further experimental studies. In the future, these results can be applied in the design and development of recombinant vaccines in the field of poultry vaccines.

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Conflict of interest

Atena Mozafari, Jafar Amani, Shahla Shahsavandi and Ali Hatef Salmanian declare that they have no conflicts of interest.

Human and Animal Rights

This article does not contain any studies with human or animal subjects.

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