Saudi Journal of Biological Sciences 29 (2022) 103283

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

In silico epitope-based vaccine design against influenza a neuraminidase protein: Computational analysis established on B- and T-cell epitope predictions

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ARTICLE INFO

Article history:
Received 29 September 2021
Revised 18 March 2022
Accepted 17 April 2022
Available online 21 April 2022

Keywords:
Influenza
Epitope prediction
Docking simulation
Toxicity
Antigen-antibody reaction

ABSTRACT

Objective: Influenza A virus belongs to the most studied virus and its mutant initiates epidemic and pandemics outbreaks. Inoculation is the significant foundation to diminish the risk of infection. To prevent an incidence of influenza from the transmission, various practical approaches require more advancement and progress. More efforts and research must take in front to enhance vaccine efficacy.
Methods: The present research emphasizes the development and expansion of a universal vaccine for the influenza virus. Research focuses on vaccine design with high efficacy. In this study, numerous computational approaches were used, covering a wide range of elements and ideas in bioinformatics methodology. Various B and T-cell epitopic peptides derived from the Neuraminidase protein N1 are recognized by these approaches. With the implementation of numerous obtained databases and bioinformatics tools, the different immune framework methods of the conserved sequences of N1 neuraminidase were analyzed. NCBI databases were employed to retrieve amino acid sequences. The antigenic nature of the neuraminidase sequence was achieved by the VaxiJen server and Kolaskar and Tongaonkar method. After screening of various B and T cell epitopes, one efficient peptide each from B cell epitope and T cell epitopes was assessed for their antigenic determinant vaccine efficacy. Identical two B cell epitopes were recognized from the N1 protein when analyzed using B-cell epitope prediction servers. The detailed examination of amino acid sequences for interpretation of B and T cell epitopes was achieved with the help of the ABCPred and Immune Epitope Database.
Results: Computational immunology via immunoinformatic study exhibited RPNDKTG as having its high conservancy efficiency and demonstrated as a good antigenic, accessible surface hydrophilic B-cell epitope. Among T cell epitope analysis, YVNISNTNF was selected for being a conserved epitope. T cell epitope was also analyzed for its allergenicity and cytotoxicity evaluation. YVNISNTNF epitope was found to be a non-allergen and not toxic for cells as well. This T-cell epitope with maximum world populace coverages was scrutinized for its association with the HLA-DRB1*0401 molecule. Results from docking simulation analyses showed YVNISNTNF having lower binding energy, the radius of gyration (Rg), RMSD values, and RMSE values which make the protein structure more stable and increase its ability to become an epitopic peptide for influenza virus vaccination.
Conclusions: We propose that this epitope analysis may be successfully used as a measurement tool for the robustness of an antigen–antibody reaction between mutant strains in the annual design of the influenza vaccine.

Abbreviations: SARS, Severe acute respiratory syndrome; *HA*, Hemagglutinin; *NA*, Neuraminidase; *HCP*, Health care personal; *H1N1*, Influenza A; *HAE*, Human airway epithelial; *pdm09*, Pandemic Disease Mexico 2009; *IEDB*, Immune Epitope Database; *KS*, Karplus & Schulz flexibility; *HLA*, Human leukocyte antigen; *IC50*, Half maximal inhibitory concentration; *MMPBSA*, Molecular Mechanics Poisson-Boltzmann Surface Area; *RMSD*, Root means square deviation; *RMSF*, Root mean square fluctuation; *Rg*, Radius of gyration; *MD*, Molecular dynamics.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103283

1. Introduction

Influenza is an extremely contagious pulmonary-related disorder triggered by influenza viruses. Influenza virus is responsible for instigating significant seasonal and pandemic morbidity and mortality across the world. Influenza virus is a major health concern issue throughout the world. Around 3 to 5 million cases of influenza have been reported that cause serious complications and 500,000 mortality cases each year (Tregoning et al., 2018). According to the reported kinds of literature, the total annual estimate for the influenza epidemic in the USA alone was over 100 billion dollars. To attenuate, the impacts of endemics and pandemics all over the world, the international community start making progress for it (Lisa, 2011). Severe acute respiratory syndrome (SARS) pandemic caused by avian influenza posed threat in many countries to devise a pandemic plan. Influenza virus can cause a serious infection in the human lung as stated (Lisa, 2011). It can trigger severe lower respiratory tract infection that results in the inflammation of alveolar regions and the development of acute respiratory distress syndrome that results in death from respiratory failure. Influenza virus has become a major threat for febrile infections while traveling (Belser et al., 2012).

Influenza viruses originate from the Orthomyxoviridae family. This virus may be further split up into four genera A, B, C, and D. Lisa in 2011 reported type A virus as a major infectious agent that may be responsible for all seasonal and pandemic influenza outbreaks. Type A viruses are distinguished further in separate subtypes and divisions is because of the location of two viral membrane glycoproteins i.e., hemagglutinin (HA) and neuraminidase (NA) (Katherine and Kanta, 2015) Studies reported H1N1 as the most epidemic infectious cause globally and has been found as the key elements of seasonal preventable influenza vaccines. Several epidemiological studies were carried out for the influenza A (H1N1) pandemic to check the frequency of infection rates and risk factors that leads to H1N1 infection among healthcare personnel (HCP). It has been reported that continuous antigenic shift of HA and NA, responsible for the development of new influenza pandemics with its therapeutic potential (Jeffery and David, 2010). Reports have confirmed the 2009 H1N1 virus being the most dangerous pandemic virus and it has affected a large population mainly children and young adults. Patients with chronic concurrent disorders and those at the extreme of age and pregnant women are at higher risks of complications (Hui et al., 2017). In 2009 about 162,000 types of more laboratory cases related to the 2009 H1N1 pandemic virus were found (Petrosillo et al., 2009). H1N1 influenza A pandemics started in March 2009. It was significantly showing the quadruple blending of the chromosomal genes of existing species from different individuals into different sequences. It has been reported in 2009 that influenza strains belonging to humans and avians were commonly observed. H1N1 influenza virus has caused seasonal epidemics along with influenza B viruses in most countries (Dotis and Roilides, 2009). Presently, research on vaccine development for influenza aims to discover new methods that allow a more complete inoculation. Lu et al in 2002 reported many epitopic sequences of distinct influenza virus strains originating from several influenza viral surface glycoproteins. These surface proteins are restricted in the most external part of a viral particle, and they are gaining a lot of interest as the best source of epitope data (Lu et al., 2002).

Numerous studies have been marked on commercial influenza vaccines targeting conserved viral protein (Jazayeri and Poh, 2019). Studies have reported antigenic epitopes as the most practical and harmless method to develop vaccines to induce the desired immune responses (Jazayeri and Poh, 2019). The epitope-driven vaccine is an innovative method efficaciously implemented in various research groups. Studies revealed vaccinated progressed targeted preserved epitopes in variable and instantaneous pathogens that have the potential to mutate (Iurescia et al., 2012). Lately, the immunological features of a "recombinant multi-epitope vaccination" were projected to be distinctive and acceptable. This recombinant multi-epitope vaccine has been successfully generated in the prokaryotic system and has been approved for *in silico* immunogenicity tests against the influenza virus (Maleki et al., 2022). In a recent work on the *in-silico* examination of local RNA secondary structure in influenza virus A, B, and C, Peterson et al in 2022 discovered indications of extensive ordered stability but minimal evidence of major covariation (Peterson et al., 2022). Based on the neuraminidase protein, a recent study presented a promising multi-epitope peptide vaccine candidate against Influenza A virus. This peptide might defend against a variety of viral subtypes and provide a broad spectrum of protection against this seasonal sickness (Behbahani et al., 2021). Antigenic epitopes have been continuously used for their therapeutic efficiency and their administration has shown protective action against various adverse reactions and diseases. They showed their imperative role in the advancement of recombinant protein-based vaccines and peptide-based immunogens (Huang, 2006). The progress of seasonal influenza virus inoculation showed a therapeutic efficacy of 75% with a decline in immunogenicity in elderly humans (Kumar et al., 2018). The development of a multivalent peptide vaccine may be beneficial that presents linear peptide "exposed" B-cell epitopic peptides arising from the different sequence of neuraminidase protein of both influenza A and B viruses. Improved vaccines induce and trigger shielding therapeutic immune responses against influenza A viruses that may embody universal influenza vaccine (Gao et al., 2013). The collective approach involves designing an epitope, consolidating molecular adjuvants, developing antigen delivery techniques, and designing some vaccination routines together to overwhelm hurdles that are being observed during the advancement of a universal influenza vaccine. The present study describes a significant advancement in this area.

Reports have confirmed the location and role of Hemagglutinin (HA) upon entry of host cell (Caroline et al., 2014). Very little research so far is being conducted on another cell surface viral protein known as neuraminidase (NA) Different over-the-counter medications for influenza have been in use because they target the active site of NA. In some reported literature, it has been observed that NA antibodies help in reducing the onset of many diseases (Skehel and Wiley, 2000). NA is reported to induce a prolonged immunity and evidenced defense against a given pathogen. Few research conducted on NA proved it as a promising marker for the imminent influenza vaccines (Skehel and Wiley, 2000). NA cleaves sialic acid enzymatically, an influenza host cell receptor, and makes it possible to release their viral particles that infect other cells. Reported literature suggested that NA in-operative virus in the occurrence of NA inhibitors makes an aggregate on the apical cell surface (Air and Laver, 1989). According to the reported literature, NA plays an important role in viral infection including mucus breakdown that allows the virus to diffuse into the respiratory tract during infection. According to studies, NA's sialidase process enables the virus's entrance into cells by acceler-

ating the disintegration of sialic acids provided by decoy receptors such mucins, thereby giving NA a pivotal role in virus assembly (Palese et al., 1974). A recent study was conducted on the involvement of influenza neuraminidase in so many phases of the virus replication cycle and proved to be a good target for vaccines and antiviral drugs. Moreover, study looked at whether NA-specific antibodies might stop A(H1N1) pdm09 from replicating in primary human airway epithelial (HAE) cells (Smet et al., 2022). The study found that H6N1 virus infection of HAE cells was reduced by human sera with NI activity against the N1 of A(H1N1) pdm09, suggesting the possible role of anti-NA antibodies in the regulation of influenza virus infection in people.

Keeping in view the importance of neuraminidase in the replication of viruses, presently the different treatments for influenza viruses targets mainly the neuraminidase enzyme activities. This technique may be inconsequential in some cases since medications that develop resistance to particular virus strains become apparent shortly after antiviral therapy. Bioinformatics analysis of entire neuraminidase sequences in GenBank TM discovered a separate preserved peptide portion in the neuraminidase protein, which is designated as HCA-2 and is fully conserved (Colman, 1992; Chun et al., 2008).

To summarize, it has been determined that Neuraminidase protects against the development of infection, and B cell epitopes contribute to the advancement of the influenza vaccination. More findings need to be validated to check the candidates for good epitope-based vaccines. For this reason, NA epitope peptides hold a good candidate (Colman, 1992; Zhu et al., 2010). After computational analysis, the vaccines need to be evaluated in preclinical studies and clinical trials in humans also. This method can develop distinct history in the field of epitope-based vaccine design.

2. Research methodology

The research methodology that was employed for the influenza vaccine strategy is summed up in Fig. 1 given below. The following diagram summarizes a vaccination strategy that selects the neuraminidase protein for vaccine development. All of the experiments were carried out on a personal computer running Windows XP Professional Edition. The experiment was also carried out with the help of the Internet and a variety of online and offline tools. Vaccines that detect distinct B and T-cell epitopic peptides extracted from the Neuraminidase protein N1 have been designed and produced using bioinformatics approaches.

In this study, *in silico* analysis was used to determine the unique B cell and T-cell epitope proteins of N1 neuraminidase that are antigenically the most significant for Influenza A virus subtypes. The antigenicity, stability, and length of several particular B and T-cell epitopes from Neuraminidase protein were chosen in this investigation. The NCBI database was used to get the full viral proteome of influenza A (H1N1) strains. The diverse immune framework approaches of the conserved sequences of N1 neuraminidase were evaluated using a variety of databases and bioinformatics tools. Amino acid sequences were retrieved from the NCBI database. VaxiJen server and Kolaskar and Tongaonkar technique were used to achieve the antigenic nature of the neuraminidase sequence. Following the assessment of numerous B and T cell epitopes, the antigenic determinant vaccination effectiveness of one efficient peptide from each B and T cell epitope was evaluated. B-cell epitope prediction servers were used to study the N1 protein, and two identical B cell epitopes were recognized. ABCPred and the Immune Epitope Database were used to examine amino acid sequences in detail for the interpretation of B and T cell epitopes.

2.1. Repossession of protein sequence

Various amino acid sequences obtained from the neuraminidase gene were collected from the pandemic influenza H1N1 strain (n = 465). Whole sequences were recovered from the NCBI database (Noronha et al.,2012). Sequences were attained from the recorded database in FASTA format.

2.2. Variability analysis of neuraminidase glycoprotein

The conservancy of repossessed sequences remained connected by applying the clustal omega program of EBI (Li et al., 2015). Multiple sequence alignment (MSA) was obtained later. The obtained MSA was scrutinized applying Jalview (Waterhouse et al., 2009).

2.3. Estimation of antigenicity of the NA protein

To check whether an assumed sequence has an antigenic nature or not, VaxiJen v2.0 server (Khan et al., 2015) and Kolaskar and Tongaonkar method was instigated (Kolaskar and Tongaonkar, 1990).

2.4. Estimation of linear B-cell epitope

To quantify the B- cell epitope, Bepi Pred 2.0 and ABC pred web servers were employed by inserting sequence into it (Sathish et al., 2018) and (Lenka et al., 2016). Bepi Pred 2.0 server analyzes only good scoring epitopes and uses a statistically hidden Markov model. ABC pred server predicts epitope with the threshold of 75% and a window length 12 amino acids (Saha and Raghava, 2006). The epitopes that were observed using above mentioned servers were surveyed. Epitopes that were distinguished mutually with the servers were taken into consideration for the next analysis.

2.5. To speculate surface accessible regions

The effective epitope is accessible to the receptor of the cell surface. For surface accessible efficacy of an epitope, Emini surface accessible prediction server ((Vita et al., 2009) was employed. The selected sequence was inserted into the server. Surface accessible epitopes were further considered for another analysis.

2.6. Evaluation for the B cell epitope conservancies

Conservancies investigation of surface accessible epitopes were evaluated using the epitope conservancy tool, IEDB (Ward et al., 2017). 80 percent threshold was kept set for sequence identity threshold.

2.7. Measurement of B-cell epitopic potential

To check the antigenic properties of individual epitopes and whole protein sequence as well. The antigenic potential was verified for B-cell epitopes using the VaxiJen v2.0 server (Doytchinova and Flower, 2007). VaxiJen server shows the antigenic nature of a protein-based on the physicochemical properties of amino acids.

2.8. To measure B antigenic determinant cell flexibility and hydrophilicity

The antigenic nature of a peptide can also be depicted by its hydrophilicity and flexibility. To quantity, the flexibility and hydrophilicity, epitopes that are conserved were measured in Kar-

Fig. 1 A graphical representation of the approaches utilized in the development of peptide vaccines.

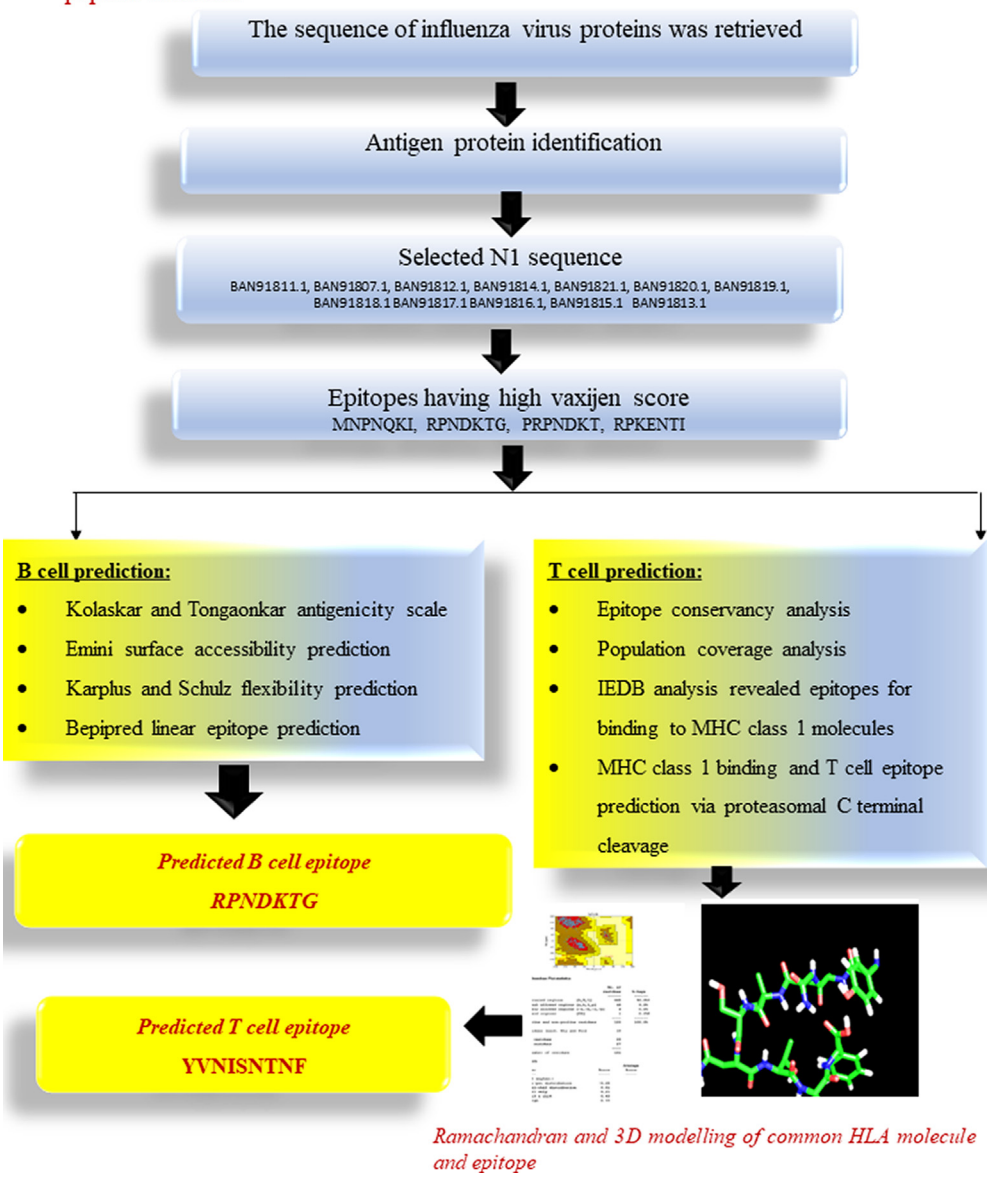


Fig. 1. A graphical representation of the approaches utilized in the development of neuraminidase target vaccine design.

plus & Schulz (KS) flexibility (Karplus and Schulz, 1985) and Parker hydrophilicity estimation tools (Parker, et al., 1986).

2.9. T Cell epitope computation and conservancy investigation

Measurement of T cell epitope is carried out using NetCTL server of IEDB (Lohia and Baranwal, 2015) and NetCTL pan methodology along with a protein sequence. The threshold of 0.50 and the sensitivity of 0.89 and the specificity of 0.94 were obtained. Estimation of proteasomal cleavage and MHC class I affinity by NetCTL gives an estimation of T cell epitopes. Based on these estimations and scoring, six epitopes were designated for more investigation. MHC-I prediction server of IEDB recognized MHC-I alleles that interact with every selected epitope. To assess the half-maximal inhibitory concentration (IC50) of MHC-I alleles binding peptides, a method known as stabilized matrix method (SMM) was incorporated. The cut-off value is 220 nM. Binding analysis requires MHC class I alleles 9 amino acid peptide length were chosen.

2.10. Measurement of T cell epitope allergenicity

To measure the allergenicity of selected T cell antigenic determinant, AllerTOP v. 2.0 reported by Utpal et al., 2018 will be applied. AllerTOP is applicable for *in silico* measurement of diverse antigens constructed basically on their physical and chemical properties of proteins. AllerTOP uses an alignment-free methodology to determine specific allergenicity which in turn depends on some properties of amino acids such as hydrophobic nature, magnitude, relative abundance, helix, and β-strand forming tendencies.

2.11. To measure the toxic nature of the T-cell antigenic determinants

Epitope utilized for efficient vaccine development should be non-toxic with high antigenicity. ToxinPred web server reported by Gupta et al in 2013 was applied for the measurement of T-cell epitope toxicity.

2.12. Population coverage interpretation of T-cell epitopes

For effective vaccine development, predicted peptide(s) may show protective action for the human population in the major broader zones. To scrutinize the world populace of different epitopes, particular T cell epitopes along with their HLA alleles that correspond to the class I and II were checked using population coverage IEDB analysis resource.

2.13. D assembly interpretation of preserved T-cell epitopic peptides and HLA-DRB1*0401

YVNISNTNF and FSFKYGNGV were chosen for simulation assay individually for each epitope and allele, the three-dimensional structure of epitopic peptide and its 3D buildings affinity of the peptides and its companionable alleles were essential. The 3D assemblies of the designated peptides were constructed applying the PatchDock. The protein structure was considered as a rigid body while ligands were completely flexible. Around 100 docking solutions were figured for each ligand keeping different parameters as a defaulting. We selected the HLA-DRB1*0401 (PDB ID:1D5M) crystal structure of MHC II in response to the influenza AH1N1 virus based on some experimental data and computational predictions. HLA-DR allele is present in most humans. Experimental analysis was carried out in which an epitope and MHC II (PDB ID: 1D5M) were crystallized together. The Patch Dock program was used. The various binding pose models were generated from patch dock results and only the highest score result was chosen as representative structures for MHC class II (HLA-DRB1*0401)-epitope complex for further analysis.

2.14. Conserved T-cell epitope docking simulation with the HLA-DRB1*0401

Molecular dynamic simulation for both the complexes was initiated for a time of 10 ns using Gromacs version (4.6.7) (Oostenbrink C, Gunsteren, 2004). The SPC 216 water model was used to solvate the system. Sodium and chloride ions were added to the system for neutralization. Undesirable steric clashes were avoided. The PRODRG server implemented gives us the topology establishment of FSFKYGNGV & YVNISNTNF ligands. Model stability developed was assessed that was established on the energy minimization and Ramachandran plot. The Ramachandran plots were created by PDB sum generate software. The trajectory files were inspected to measure the value of RMSD, RMSF, and radius of gyration.

The binding free energies of each simulated system were calculated by the MMPBSA approach. MMPBSA is the normal method to compute the binding energy between protein–ligand complexes (Wang et al., 2018). The g_mmpbsa tool was used for the calculation. The binding energy between two entities was calculated by the equation1 as:

$$\Delta G = < E_{MM} > + < \Delta G_p > + < \Delta G_{ap} > - T < \Delta S > \quad (1)$$

where,

E_{MM} = molecular mechanic energy in the vacuum state.
ΔS = the entropic contribution.
ΔGp = polar solvation energy.
ΔGap = non-polar solvent energies.
T = Temperature (300 K).

3. Results

3.1. Retrieval of influenza viral protein sequence

The entire viral proteome of influenza A (H1N1) strains was obtained from the NCBI database. All the sequences were from Sendai. The lengths of these sequences ranged from 1aa to 465aa. (Accession of different genotypes: BAN91821.1, BAN91820.1, BAN91819.1, BAN91818.1, BAN91817.1, BAN91816.1, BAN91815.1, BAN91814.1, BAN91813.1, BAN91812.1, BAN91811.1, BAN91809.1, BAN91808.1, BAN91807, BAN91806). Sequences were collected in FASTA format.

3.2. Neuraminidase N1 conservancy

The neuraminidase sequence that we choose was 465 amino acid long chain. To envisage the level of the conservancy, MSN configuration and protein variability analysis was achieved. Neuraminidase was preserved in a maximum of 12 strainings. Non-conserved and partially conserved sequences were removed. Among sequences, we choose the following sequences with their BAN numbers for further analysis based on their conservancy, BAN91811.1, BAN91807.1, BAN91812.1, BAN91814.1, BAN91821.1, BAN91820.1, BAN91819.1, BAN91818.1 BAN91817.1 BAN91816.1, BAN91815.1 BAN91813.1.

3.3. Neuraminidase is antigenic in nature

To check the antigenicity of neuraminidase protein, the N1 sequence with accession number BAN91812.1 was assessed for its antigenic nature. Implementation of vaxijen server proved N1 as a possible antigen with a value of 0.5303. For virus antigenicity determination, the chosen threshold was 0.4. The window size chosen was 7 amino acids. Kolaskar & Tongaonkar antigenicity prediction tool ((Kolaskar and Tongaonkar, 1990) identified the antigenicity of N1 protein. This confirmed our selected protein to be potentially immunogenic (Fig. 2.).

3.4. MNPNQKI, RPNDKTG, PRPNDKT, RPKENTI are probable B-cell epitopes

The probable B cell epitopes are ordered as per their score. The maximum score shows a good possibility for epitopic efficiency. Table. 1 showing all peptides values above than threshold value.

3.5. MNPNQKI, RPNDKTG, PRPNDKT, and RPKENTI found similar in whole sequences

Conservancy analysis for four B cell epitopes was assessed by using the IEDB conservancy analysis tool. The entire epitopes were similar in the N1 protein sequence (Table 2).

3.6. MNPNQKI, RPNDKTG, and PRPNDKT are highly antigenic

The three probable antigens that are being recognized by the VaxiJen server are **MNPNQKI, RPNDKTG and PRPNDKT** having 0.7068, 1.0187 and 1.4942 values, respectively. The antigenic threshold was 0.4. The remaining nonefficient epitopes were removed.

3.7. RPNDKTG is flexible and hydrophilic

Flexibility and accessibility are important for the induction of immune strength. Among the three epitopes, RPNDKTG proved to have good flexibility (Fig. 3A). RPNDKTG and PNDKTGK peptides

Fig. 2.

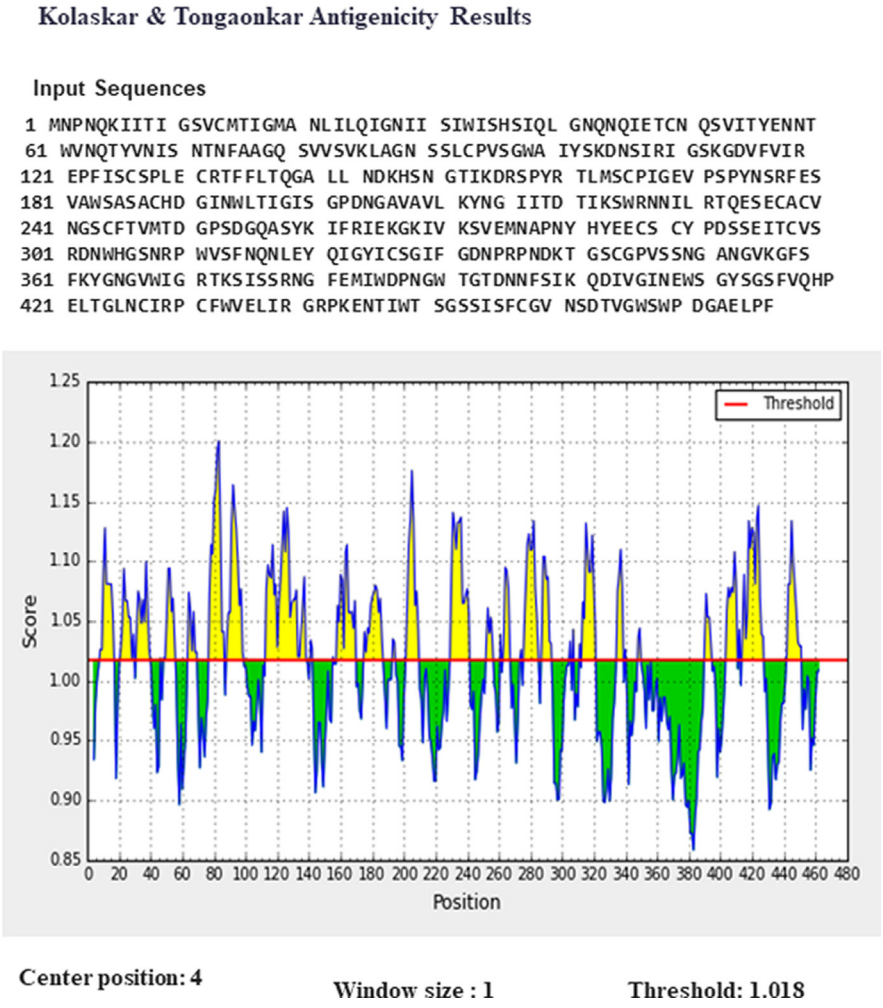


Fig. 2. Kolaskar and Tongaonkar antigenicity results confirm N1 protein antigen. The threshold value is 1.018. The residues in yellow regions have antigenic.

had good flexibility above threshold 0.991 keeping a window size of 7 amino acids and center position as 4. RPNDKTG was found to be hydrophilic also in nature and value above threshold came as 6.543 (Fig. 3B) keeping window size of 7 amino acids and center position as 4 (Table 3).

Table 1
Predicted B cell epitopes along with their scores. A higher score represents a high affinity for an epitope.

Rank	Sequence	Start position	Score
1	MNPNQKI	1	0.934
2	RPNDKTG	327	0.899
3	PRPNDKT	326	0.926
4	RPKENTI	430	0.936

Predicted B cell epitopes with their respective scores. A higher score represents a high affinity for an epitope. The length of the sequence is 465. The number of isomers from the input sequences are 454 and the threshold setting is 0.75.

3.8. T-cell epitopes, TCNQSVITY, ITYENNTWV, YVNISNTNF, FSFKYGNGV, YSKDNSIRI, CSPLECRTF, were found which were highly conserved

Different parameters chosen were set according to the requirement. NetCTL server identified several potential T cell epitopes. Based on high scoring efficiency and conservancy only 6 epitopes were chosen among all mentioned epitopic peptides. MHC I alleles intermingle comfortably with the chosen six epitopes and were analyzed by using an MHC-I binding prediction server. IC50 cutoff values was 200 [38]. N1 protein sequence was submitted in the

Table 2
Evaluation of the conservancies of B cell epitopes. The study of predicted B cell epitopes and Emini surface peptides are assessed by the IEDB conservancy analysis tool.

Epitope #	Epitope chain	Epitope length	Protein chain in percentage that matches at identity <= 100%	Lowest uniqueness	Highest uniqueness
1	MNPNQKI	6	100.00% (13/13)	100.00%	100.00%
2	RPNDKTG	6	100.00% (13/13)	100.00%	100.00%
3	PRPNDKT	6	100.00% (13/13)	100.00%	100.00%
4	RPKENTI	6	100.00% (13/13)	100.00%	100.00%

Evaluation of the conservancies of B cell epitopes. The highest uniqueness noticed in four epitope sequences (MNPNQKI, RPNDKTG, PRPNDKT and RPKENTI.

Fig.3.

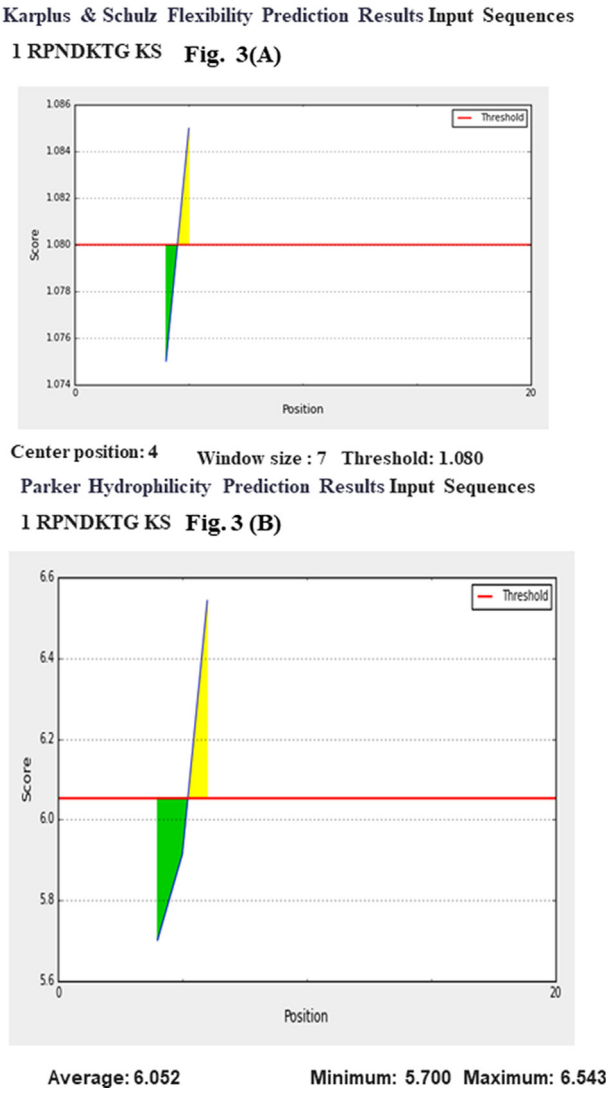


Fig. 3. (A). Flexible RPNDKTG KS epitope. The amino acid residues of the RPNDKTG KS epitope have a threshold level of 1.080. Fig. 3**. (B)** Hydrophilic nature of RPNDKTG KS epitope. Amino acid residues of the RPNDKTG KS epitope are hydrophilic in nature. Residues that are above the cutoff value (6.01, horizontal red line) are in the yellow region.

IEDB MHC class II binding prediction tool. Among all epitopes selected, only those T cell epitopes were selected that have an efficient overlapping efficiency (Table 4 and 5).

3.9. YVNISNTNF, FSFKYGNGV are not allergens

To check whether an epitope is an allergen or not, AllerTOP v. 2.0 server was applicable. In all epitopes, only YVNISNTNF and FSFKYGNGV were found to be non-irritants. Rest epitopes that were showing allergenicity were removed (Table 6).

Table 3
Predicted peptides with their possible flexibility. Values given are above threshold (0.991).

No	Sequence	Position	Length	Score
1	RPNDKTG	4	7	1.075
2	PNDKTGK	5	7	1.085

Predicted peptides with their possible flexibility. RPNDKTG is found flexible and found to be hydrophilic also in nature and value above threshold came as 6.543.

Table 4
T cell epitopes that were chosen from the NetCTL server based on the maximum combinative scores along with their conservancy.

Epitope seq	Allele	IC50 (nM)
YVNISNTNF	HLA-A*02:50	60.98
	HLA-A*26:02	132.23
	HLA-A*23:01	850.02
YSKDNSIRI	HLA-A*02:50	32.38
	HLA-A*02:17	81.78
	HLA-A*02:11	720.00
ITYENNTWV	HLA-A*02:12	119.59
	HLA-A*02:01	157.80
	HLA-A*02:17	248.12
FSFKYGNGV	HLA-A*02:19	33553667.76
TCNQSVITY	HLA-A*02:17	1269.58
	HLA-A*26:02	2926.31
	HLA-A*01:01	4023.00
CSPLECRTF	HLA-A*02:50	134.65
	HLA-A*02:17	606.26
	HLA-A*24:02	1391.97

T cell epitopes that were chosen from NetCTL server based on the maximum combinative scores along their conservancy. Conservancy is the most important criterion of an epitope to consider it for vaccine development. Six epitope sequencing showed good prediction scores and conservancy.

3.10. YVNISNTNF and FSFKYGNGV are not toxic

To measure the toxic profile of the epitopes, ToxinPred server was applicable (Gupta et al., 2013). YVNISNTNF and FSFKYGNGV epitopes are not toxic and it may be evident that these epitopes proved a potential vaccine candidate (Table. 7).

3.11. YVNISNTNF and FSFKYGNGV have maximum world populace

T-cell epitopes were evaluated for the world populace using the Population Coverage analysis tool of IEDB. Maximum epitopes had a population coverage between 26 % and 65 % when considered MHC class I and II alleles (Table 8). YVNISNTNF and FSFKYGNGV were chosen for the generation of 3D models by the PEPFOLD server

3.12. 3D structure analysis of chosen epitope YVNISNTNF and FSFKYGNGV along with MHC class II (HLA-DRB1*0401) allele

Molecular docking simulation assay requires the three-dimensional structure analysis of YVNISNTNF and FSFKYGNGV along with *HLA-DRB1*0401*. The server that was used is the PEPFOLD Peptide Structure Prediction server. Epitopes were designed to intermingle with MHC class II (HLA-DRB1*0401). The favorable interactions were made by the epitope YVNISNTNF and FSFKYGNGV at the binding site. The frequency of favorable interactions was seen maximum in epitope YVNISNTNF depicting its more stability and binding affinity towards vaccine development (Figs. 4 & 5).

Table 5
Eligible T-cell overlying antigenic determinants amongst MHC I and MHC II requisite estimation.

Epitope	MHC- I Allele	Peptide Sequence	MHC-II Allele
YVNISNTNF	HLA-A*02:50	NQTYVNISNTNFAAG	HLA-DRB1*04:01
	HLA-A*26:02	VNQTYVNISNTNFAA	
	HLA-A*23:01	WVNQTYVNISNTNFA	
		QTYVNISNTNFAAGQ	
		WVNQTYVNISNTNFA	HLA-DRB1*07:01
		VNQTYVNISNTNFAA	
		NQTYVNISNTNFAAG	
		QTYVNISNTNFAAGQ	
YSKDNSIRI	HLA-A*02:50	AIYSKDNSIRIGSKG	HLA-DRB1*04:01
	HLA-A*02:17	WAIYSKDNSIRIGSK	
	HLA-A*02:11	GWAIYSKDNSIRIGS	
		SGWAIYSKDNSIRIG	
		VSGWAIYSKDNSIRI	
		YSKDNSIRIGSKGDV	HLA-DRB1*13:02
ITYENNTWV	HLA-A*02:12	QSVITYENNTWVNQT	HLA-DRB1*04:04
	HLA-A*02:01	NQSVITYENNTWVNQ	
	HLA-A*02:17	CNQSVITYENNTWVN	
FSFKYGNGV	HLA-A*02:19	GVKGFSFKYGNGVWI	HLA-DRB1*07:01
		VKGFSFKYGNGVWIG	
		KGFSFKYGNGVWIGR	
		KGFSFKYGNGVWIGR	HLA-DRB5*01:01
		VKGFSFKYGNGVWIG	
		FSFKYGNGVWIGRTK	
		NGVKGFSFKYGNGVW	
TCNQSVITY	HLA-A*02:17	NQIETCNQSVITYEN	HLA-DRB4*01:01
	HLA-A*26:02	IETCNQSVITYENNT	
	HLA-A*01:01	QIETCNQSVITYENN	
		TCNQSVITYENNTWV	
		ETCNQSVITYENNTW	
		NQIETCNQSVITYEN	HLA-DRB1*04:01
CSPLECRTF		PFISCSPLECRTFFL	HLA-DRB1*15:01
		FISCSPLECRTFFLT	HLA-DRB4*01:01
		ISCSPLECRTFFLTQ	HLA-DRB1*11:01
		SCSPLECRTFFLTQG	HLA-DRB1*04:04
		ISCSPLECRTFFLTQ	

Eligible T-cell overlying antigenic determinants amongst MHC I and MHC II requisite estimation. Epitopes showed good efficiency in overlapping between MHC I and MHC II binding predictions.

Table 6
Prediction of allergenicity of epitopes.

YVNISNTNF	Your sequence is: PROBABLE NON-ALLERGEN The nearest protein is UniProtKB accession number P58514 defined as non-allergen.
FSFKYGNGV	Your sequence is PROBABLE NON-ALLERGEN. The nearest protein is UniProtKB accession number P86001 defined as non-allergen.
YSKDNSIRI	Your sequence is: PROBABLE ALLERGEN The nearest protein is: UniProtKB accession number P04122 defined as an allergen
ITYENNTWV	Your sequence is PROBABLE ALLERGEN. The nearest protein is NCBI gi nimber 2,392,604 defined as an allergen.
TCNQSVITY	Your sequence is: PROBABLE ALLERGEN The nearest protein is: UniProtKB accession number P35760 defined as an allergen
CSPLECRTF	Your sequence is PROBABLE ALLERGEN. The nearest protein is: UniProtKB accession number Q9S915 defined as an allergen

Prediction of allergenicity of epitopes. T cell epitopes that proved non-allergens can have a good efficiency for vaccine design.

*3.13. T cell epitopes YVNISNTNF have lower binding energy than epitope FSFKYGNGV bound with the HLA-DRB1*0401 allele confirming its more stability*

The binding free energy for epitope YVNISNTNF and FSFKYGNGV were analyzed by means of the MM/PBSA method (Table 9). MM-PBSA calculations for the protein–ligand complex were performed on the last 10-ns trajectories by using g_mmpbsa tool embedded in GROMACS software. The snapshots of the HLA-DRB1*0401 – YVNISNTNF complex derived from MD simulation trajectories were taken from 6000 ps to 8000 ps at every 15 ps for MM/PBSA calculations. Similarly, coordinates of HLA-DRB1*0401 – FSFKYGNGV were taken from 4000 ps to 6000 ps at every 15 ps. Among them, only the epitope YVNISNTNF properly occupied the receptor and has the lowest binding energy of −211.596 than the second epitope FSFKYGNGV having binding energy −308.629. The smallest binding energy of the epitope signifies the greatest binding affinity and is effective for vaccine development. To further justify the quality and reliability of the epitope YVNISNTNF, Ramachandran plots analysis was applied and PDBsum generates tool was used. Ramachandran plot showed that 90% of residues are in the favorable region, 9.2% were found in allowed regions, and 0.2% in disallowed regions (Fig. 6). A good model represents 90% in the favored regions. Therefore, the epitope YVNISNTNF was considered an efficient epitopic peptide for vaccine development. Another epitope FSFKYGNGV was eliminated for molecular dynamics analysis.

3.14. Molecular dynamics simulation of epitope YVNISNTNF and FSFKYGNGV predicted epitope YVNISNTNF forming a stable protein complex

The root means square deviation (rmsd), root mean square fluctuation (rmsf), the radius of gyration (Rg) analysis were completed using gromacs 5.0 package. The radius of gyration found for the docked complex exhibited that the distance in rotating complex from the center of mass is 2.95 nm that decrease up to 2.9 nm at the time duration of 800 picoseconds (Fig. 7 D). The RMSD value obtained was 0.45 nm (Fig. 7. A, B) while as RMSF score obtained was 0.1 nm (Fig. 7 C). These scores give us strong complex stability.

4. Discussion

Influenza is the most prevalent infectious respiratory disease that can result in serious health problems. Influenza is highly transmissible in nature and has demonstrated rapid transmission. Because of the relative involvement of epidemic influenza transmission dynamics, heritable viral genetic changes, and environmental variations, influenza viruses are known to generate a significantly high risk of sickness and death worldwide (Monto, 1987). Currently, influenza vaccinations are designed to produce specific neutralizing antibodies that protect cells from pathogenic pathogens by neutralizing whatever effects they may have. Research is being carried out to uncover some approaches for the production of a more comprehensive influenza vaccine (Damian and Ian, 2012). Ekiert et al., 2009 reported various sequences of

Table 7
Measurement of Toxicity of selected non-allergenic epitopes.

Peptide ID	Peptide Sequence	SVM Score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt.
	FSFKYGNGV	−1.08	Non-Toxin	0.01	−0.06	−0.59	1.00	1018.26
	YVNISNTNF	−1.47	Non-Toxin	0.05	−0.20	−0.84	0.00	1071.28

Measurement of toxicity of selected non-allergenic epitopes. Epitope with peptide sequence FSFKYGNGV and epitope with peptide sequence YVNISNTNF may have preventive and beneficial functions in vaccine development.

Table 8
Selected epitopes with population coverage.

Epitope Sequence	% Individuals	Cumulative % population coverage
YVNISNTNF	94.36	100
FSFKYGNGV	5.64	5.64

Selected epitopes with population coverage. YVNISNTNF and FSFKYGNGV epitopes showed ideal population coverage with a percentage of 94% and 5.64%.

epitopic peptides of influenza that emerge viral surface glycoproteins. Because these proteins are involved in the formation of virus attachment to the host cell, they could be the source of epitope data.

We looked at how far neuraminidase-based B and T-cell epitopic peptides vaccines have progressed in terms of stimulating a good antibody response against influenza subtypes (H1N1). A unique amino acid sequence moiety was discovered to recognize B cell and T cell epitopes. Affinity analysis and molecular docking

Fig. 4. (I)

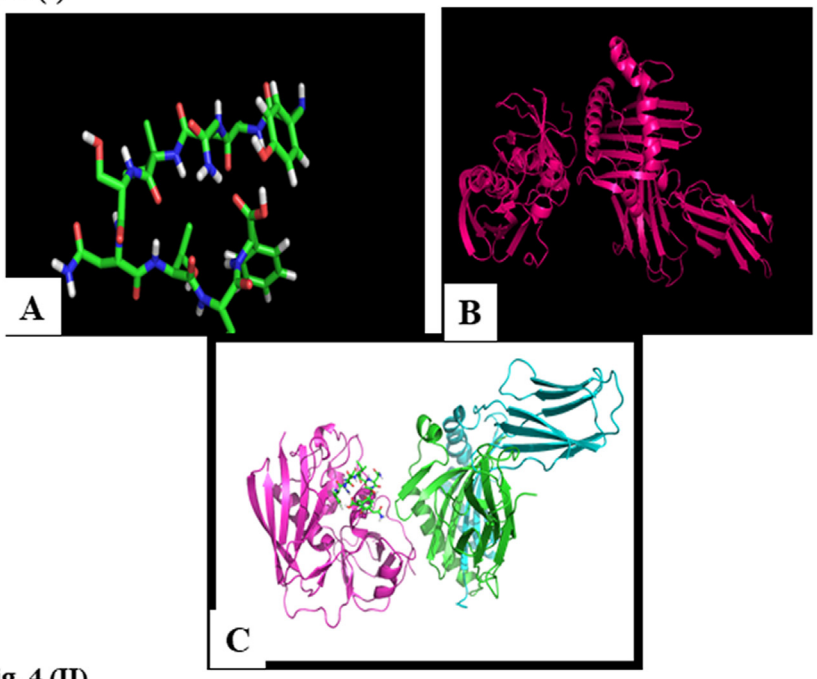


Fig. 4 (II)

Number of Favourable Interactions (YVNISNTNF)

TYR1	
TRP197(Chain C)	27
GLN3(Chain C)	12
TYR198(Chain C)	9
GLU10(Chain C)	2
ASP199(Chain C)	2
VAL2	
GLU10(Chain C)	1
ASN3	
HIS12(Chain C)	5
MET216(Chain C)	1
TYR213(Chain C)	1
ASP199(Chain C)	1
ILE4	
HIS12(Chain C)	2
SER5	
ALA203(Chain C)	8
LYS212(Chain C)	8
TYR213(Chain C)	5
ASN6	
LYS212(Chain C)	37
ASP209(Chain C)	6
THR7	
MET215(Chain C)	2
ASN8	
MET215(Chain C)	17
MET216(Chain C)	10
ASN218(Chain C)	5
LYS69(Chain C)	1
PHE9	
MET216(Chain C)	5

Fig. 4. (I). (A) Structure of predicted epitope "YVNISNTNF", (B) Structure of predicted HLA-DRB1*0401" (C) Docking of YVNISNTNF with HLA-DRB1*0401, docking interaction was visualized with the chimera, version 1.11.2. Fig. 4. **(II).** The favorable interactions made by the epitope (YVNISNTNF) at the binding site are tabulated above. The highlighted green shows the frequency of favorable contacts or interactions of more than 10 made by the epitope (YVNISNTNF) and MHC II (HLA-DRB1*0401) protein residues.

Fig. 5(I)

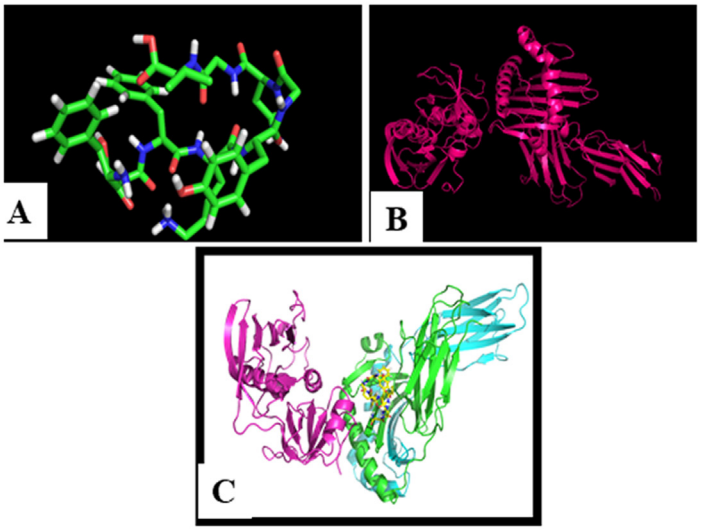


Fig. 5 (II)

Number of Favourable Interactions (FSFKYGNGV)

PHE1	
GLU134(Chain A)	12
SER2	
ASP35(Chain A)	10
ALA37(Chain A)	3
TYR46(Chain C)	2
MET36(Chain A)	1
PHE3	
GLY20(Chain A)	12
TYR46(Chain C)	10
SER19(Chian A)	1
MET36(Chain A)	1
LYS4	
TYR46(Chain C)	11
LYS71(Chain C)	1
TYR5	
No interactions	
GLY6	
VAL116(Chain A)	1
ASN7	
LYS71(Chain C)	15
ASP17(Chain A)	9
VAL116(Chain A)	1
GLY8	
VAL116(Chain A)	2
VAL9	
PHE137(Chain A)	6
VAL117(Chain A)	2
GLU134 (Chain A)	1

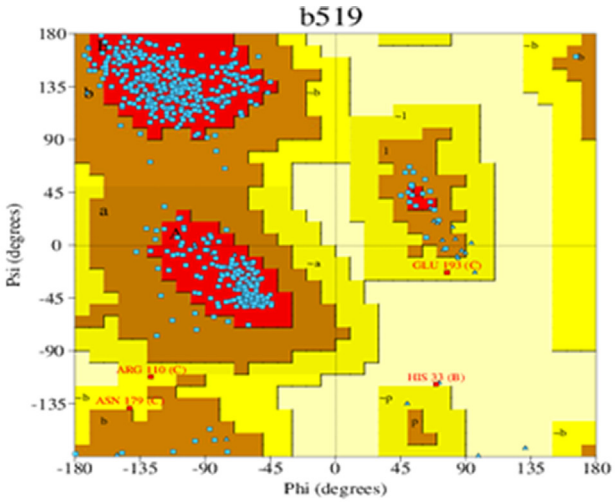
Fig. 5. (I). (A) Structure of predicted epitope "FSFKYGNGV", (B) Structure of predicted HLA-DRB1*0401" (C) Docking of FSFKYGNGV with HLA-DRB1*0401, docking interaction was visualized with the chimera, version 1.11.2. Fig. 5**. (II).** The favorable interactions made by the epitope (FSFKYGNGV) at the binding site are tabulated above. The highlighted green shows the frequency of favorable contacts or interactions of more than 10 made by the epitope (FSFKYGNGV) and MHC II (HLA-DRB1*0401) protein residues.

Table 9
Comparison of binding energies of two Epitopes.

	Van der Waal Energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	Non-polar solvation energy (kJ/mol)	Binding energy (kJ/mol)
YVNISNTNF	−222.144	−129.170	165.552	−25.834	−211.596
FSFKYGNGV	−312.466	−891.379	931.204	−35.988	−308.629

Comparison of the binding energy of two Epitopes. YVNISNTNF may be considered as a possible T-cell epitope for the vaccine development.

were carried out. Antibodies that bind to the neuraminidase glycoprotein clump virus on the cell surface, preventing it from being released from an infectious cell. Several antiviral medicines have been reported that target sialic acid-binding and inhibit neuraminidase enzyme activity. Neuraminidase, like Haemagglutinin, has been reported to be an effective inducer of cross-protective



1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	468	90.0%*
Additional allowed regions	[a,b,l,p]	48	9.2%
Generously allowed regions	[~a,~b,~l,~p]	3	0.6%
Disallowed regions	[XX]	1	0.2%*
Non-glycine and non-proline residues		520	100.0%
End-residues (excl. Gly and Pro)		10	
Glycine residues		28	
Proline residues		27	
Total number of residues		585	

2. G-Factors

Parameter	Score	Average Score
Dihedral angles:-		
Phi-psi distribution	-0.28	
Chi1-chi2 distribution	0.01	
Chi1 only	0.21	
Chi3 & chi4	0.43	
Omega	0.49	

Fig. 6. Ramachandran Plot of predicted model for the neuraminidase protein of influenza virus along with statistics showing 90.0%, 9.2%, and 0.2% of the protein residues in preferred, permissible, and forbidden areas individually and the G-factor for the model.

immunity (Sathish et al., 2018). Based on these findings, we can conclude that an epitopic peptide vaccine targeting conserved neuraminidase protein domains may boost the protective and robust immune response.

In this study, we used various bioinformatics tools and characteristics to distinguish various epitopes that are conserved in influenza strain virus. Computational determination looks to be particularly cost-effective in the manufacturing of epitopes and

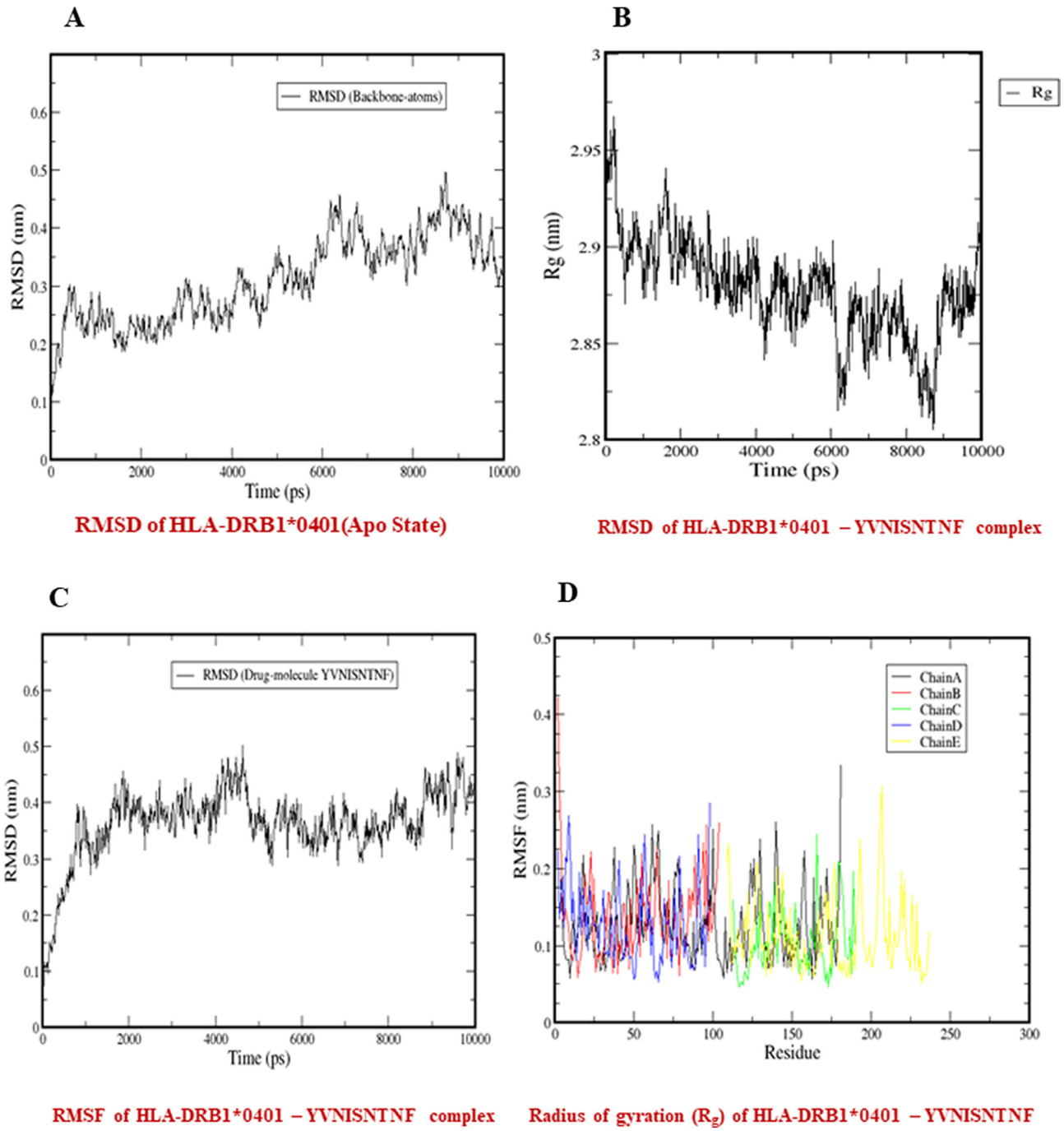


Fig. 7. Molecular dynamics simulation of the vaccine candidate **(A)** Rg plot; vaccine concept is constant in its compressed system throughout the simulation time. High Rg means lower protein stability. Rg value of this protein structure is between 2.85 and 2.95. **(B)** The RMSD trajectory is used to predict the stability of the protein. A higher RMSD value implies low stability of the protein structure. The average RMSD trajectory value for the MHC-II allele ranges between 0.2 nm and 0.4 nm. The RMSD value for the epitope is in-between 0.1 and 0.45. **(C)** RMSF; RMSF-Root Mean Square Fluctuation plot, peaks show the regions with high flexibility. The RMSF range of the mutant protein structure is between 0.1 nm and 0.3 nm.

peptide vaccines (Hua et al., 2017). Epitopic peptides can identify B-cell and T-cell antigenic determinants which triggers distinct immune response (Patronov and Doytchinova, 2013). In our study, epitopic peptides that induces an immune response for mutually B ell and T cell antigenic determinants were analyzed. B-cell epitope recognition has a significant application in immunodetection and immunotherapy. Epitope even in the small immune unit may be effective enough to produce a strong humoral immune response without any detrimental change to humans (Sun et al., 2013). The majority of vaccines are established based on B cell immunity. Although T cell epitope-based vaccines also show more attention and interest against various common viruses (Zheng et al., 2017). We observed some extremely conserved B-cell and T-cell epitopes that can act as universal vaccines to protect against various influenza-based infections. Using in-silico methods and the neuraminidase N1 inner protein, we attempted to create an influenza vaccine. The viral envelope contains the NA protein. We looked at the conservation of the N1 protein and discovered that 12 strains of neuraminidase have a 90 percent conservation rate among the 20 N1 protein strains in the NCBI database. The neuraminidase N

1protein has no antigenic determinants that have been identified. This study aims to produce an epitopic-based peptide vaccine against the influenza virus, which could be a significant step forward in vaccine development.

Recognizing highly conserved sequences is critical for improving the epitopic peptide-based vaccination concept. MSA analysis and an entire unpredictability site study were used to establish the progression of N1 protein conservation. They revealed that N1 protein is significantly conserved in all influenza strains. These findings are in line with previous research that suggests influenza's persistence and diversity. A candidate for epitope-based vaccinations based on H1N1 HLA-restricted T cells (Tan et al., 2010). A protein must have an antigenic response to elicit a sufficient immune response in order to be considered an active vaccination candidate. VaxiJen server the antigen property of an N1 protein that is already stated by Doytchinova and Flower, 2007. Our results confirmed the antigenic nature of the conserved sequences by the implementation of these software's. These results are based on the previous findings in which various B cell epitope prediction methods had been established (El-Manzalawy et al., 2010). Diverse B-cell epitope estimation procedures were implemented to check the immune response. MNPNQKI, RPNDKTG, PRPNDKT and RPKENTI were four epitopes that were seen common in all prediction servers chosen for more advanced analysis. B-cell epitopes should have affinity to bind to antibodies to elicit an immune response. B-cell epitopic peptides play a significant role in peptide inoculation development and in diagnosing communicable diseases. For the characterization of different epitopes, experiments based on different methodologies were time-consuming and expensive. Conserved epitopes served as a better protective agent for different strains for vaccine development comparatively highly flexible genomic regions. For the manufacturing of epitope vaccines, the first important thing to be noted is that epitope should be conserved. In our research study, all epitopes that we studied are highly conserved. The Conservancy of respective epitopes was calculated by using the IEDB conservancy analysis tool. B-cell epitopic peptides were verified for their antigen nature using the VaxiJen v2.0 server (Sakib et al., 2014). MNPNQKI, RPNDKTG, and PRPNDKT are antigens with scores 0.7068, 1.0187, and 1.4942 respectively keeping 0.4 default parameter for threshold. Our findings were backed up by subsequent studies that showed a computational strategy for designing epitopic peptide vaccines that might induce antibody-mediated and cell-mediated immunity (Adhikari et al., 2018).

To check the protein prediction accuracy and flexibility the Karplus and Schulz flexibility scales were applicable and the calculation was performed with a threshold setting of 0.991. The hydrophilic nature of B- the cell epitope was measured using the parker hydrophilicity scale. RPNDKTG epitope was found extremely flexible in Karplus and Schulz flexibility prediction analysis (Karplus and Schulz, 1985) and extremely hydrophilic in the Parker hydrophilicity prediction tools (Parker et al., 1986). After further investigation, we discovered that RPNDKTG can be used as a B-cell epitope to assess immunological affinity and can be a useful tool for vaccine development.

T-cell epitope prediction server NetCTL was used to predict CTL epitopes in protein sequences. Various CD8 + epitopes were identified using this site. Based on prediction scores and conservancy, only the first six epitopic peptides are preferred after analysis. Cytotoxic T-cells activation needs the most efficient epitopes to get bound to major histocompatibility (MHC) class I molecules (Trowsdale and Knight, 2013). MHC alleles get bound to peptide fragments that are derived from pathogens so that they should be recognized by the appropriate T cells (Trowsdale and Knight, 2013).

The binding efficiency of MHC-I alleles with the first six chosen epitopes was checked using a stabilized matrix (SMM). Our results showed that MHC I alleles interacted with the chosen Tcell epitopes and prediction was based on IC50 cutoff (200) using the IEDB MHC class I binding prediction tool. MHC II alleles also interacted with epitopes that were retrieved from the N1 protein sequence and prediction was evaluated by using the IEDB MHC class II binding prediction tool. IC50 cutoff values were kept around 100. Finally, only those epitopes were selected for further analysis that showed overlapping between MHC I and MHC II binding predictions. These results were supported by Adhikari et al., 2018 showing the most efficient binding sites for predicted epitopic peptides against Polyprotein of oropouche virus. In our study selected peptides were TCNQSVITY, ITYENNTWV, YVNISNTNF, FSFKYGNGV, YSKDNSIRI, CSPLECRTF. AllerTOP v.2.0 served predicted out of the six epitopes, only two epitopes (YVNISNTNF and FSFKYGNGV) as non-allergens and the rest of the other remaining epitopes as potential allergens to humans. Adhikari et al in 2018 also identified non-allergen efficient T cell epitopes for vaccine design.

Peptide vaccines proved to be an emerging and promising therapeutic tool for many lethal ailments (Thundimadathil, 2012). A major constraint that disturbs the effectiveness of peptides for vaccine developments is the toxicity associated with them. The toxic effect was evaluated for two selected t cell epitopic peptides using the ToxinPred server (Zheng et al., 2017). Result analysis showed both epitopes as nontoxic. These epitopes may have prophylactic and therapeutic applications in vaccine development (Testa and Philip, 2012). To get population coverage, predicted T cell epitopic sequences with their matching MHC HLA alleles were analyzed using the population coverage analysis tool of IEDB. YVNISNTNF and FSFKYGNGV epitopes showed ideal population coverage in different geographic regions globally with a percentage of 94% and 5.64%. The 3D structures of two epitopes were chosen for docking purposes as a novel vaccine candidate for the influenza virus.

The three-dimensional shape of the epitopes and MHC molecule is required to begin the docking process. The Patch Dock program created YVNISNTNF and FSFKYGNGV epitopes with a three-dimensional structure. The intermolecular interactions between the epitopes and the HLA-DRB1*0401 allele was investigated using the Patch dock tool. After docking, the binding energies of the YVNISNTNF and FSFKYGNGV epitopes were found to be −211.596 kcal/mol and −308.629 kcal/mol, respectively. The ability of T-cell epitopes to dock to the allele is confirmed by the lower binding energy of YVNISNTNF. Following the analysis, YVNISNTNF could be evaluated as a potential T-cell epitope for vaccine development. Single letters code for Y as Tyrosine, V as Valine, N as Asparagine, I as Isoleucine, S as Serine, T as Threonine, and F as Phenylalanine in the YVNISNTNF epitope. We used a docking simulation study to create a stable MHC II–epitope complex by generating a complex of epitope–MHC II and causing several critical interactions.

In some recent surveys molecular docking studies has been approved to find out constant isotopes chosen for the MHCII allele (Antunes et al., 2018). To examine and see the interaction amongst predictable epitope and MHC II, RMSD values were analyzed. Results signify that viral protein NA tends to reach convergence at 10 ns and persisted balanced during the rest of the MD simulations. RMSD value also displayed the same comportment throughout the MD simulations. Observed RMSF values further validate the stability of the proteins. The root means square deviation (rmsd) was employed to enumerate the constancy of protein before and after docking with ligands in the simulation process. The per residue root means square fluctuation (rmsf) was used to distinguish the regions of higher flexibility in protein, was calculated as shown in Fig. 6 C. The radius of gyration of protein with ligand has been found to be in a steady-state after 50 ns which indicates the stability of models. The stability of the model was further validated by numerous structure assessment methods including the Ramachan-

dran plot. Ramachandran's plot showed good consistency of the predicted 3D models. Analysis of 3D structures will help in the identification of binding sites and may lead to the designing of new vaccines.

5. Conclusion

This study displayed effective epitopes for their application in designing a universal epitope-based peptide vaccine for entire pathogenic strains of the influenza Virus. The study helps in claiming either a B-cell or a T-cell epitope for the epitope-based peptide vaccine against the N1 Neuraminidase protein of the influenza's virus. After examination, T cell epitopic peptides revealed a high affinity for MHC class II alleles and may activate a cell immunological response. Experimental validation is required to confirm the proposed vaccine construct's safety and immunogenicity characteristics. These epitopes for vaccine development could be tested further in preclinical and clinical trials. According to the findings of the computational analysis, RPNDKTG is thought to be a B-cell epitope, while YVNISNTNF is thought to be a T-cell epitope.

6. Authors' contributions

All contributors equally contributed to and supported the research article's correct structure. The research article was drafted and revised properly. The head of the research group, Dr. Shaia Almalki, gave final approval for it to be submitted. Dr. Shaia Almalki designed the computational study and provided a clear concept. Dr. Naseem Akhter assisted with the data collection. The manuscript was revised with the help of Dr. Read A. Alharbi. Dr. Saba Beigh did the data analysis. Finally, Dr. Naseem Akhter, Dr. Saba Beigh, and Dr. Read A. Alharbi collaborated on the data interpretation under the supervision of Dr. Shaia Almalki.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The Authors are grateful to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number MOE-BU-1-2020.

References

Adhikari, U.K., Tayebi, M., Rahman, M.M., 2018. Immunoinformatics Approach for Epitope-Based Peptide Vaccine Design and Active Site Prediction against Polyprotein of Emerging Oropouche Virus. J. Immunol. Res. 8 (2018), 6718083.
Air, G.M., Laver, W.G., 1989. The neuraminidase of influenza virus. Proteins 6 (4), 341–356.
Antunes, D.A., Devaurs, D., Moll, M., Lizee, G., Kavraki, L.E., 2018. General Prediction of Peptide-MHC Binding Modes Using Incremental Docking: A Proof of Concept. Sci. Rep. 8 (1), 4327.
Behbahani, M., Moradi, M., Mohabatkar, H., 2021. In silico design of a multi-epitope peptide construct as a potential vaccine candidate for Influenza A based on neuraminidase protein. In Silico Pharmacol. 11;9 (1), 36.
Belser, J.A., Gustin, K.M., Maines, T.R., Jackwood, M.J.P., Katz, J.M., Tumpey, T.M., 2012. Influenza virus respiratory infection and transmission following ocular inoculation in ferrets. PLoS Pathog. 8, (3) e1002569.
Caroline, M., Mair, K., Andreas, H., 2014. Receptor binding and pH stability. How influenza A virus hemagglutinin affects host-specific virus infection. Biochimica Biophysica Acta (BBA) –. Biomembranes 1838 (4), 1153–1168.
Chun, S., Li, C., Domselaar, G.V., Wang, J., Farnsworth, A., Cui, X., Rode, H., Cyr, T.D., He, R., Li, X., 2008. Universal antibodies and their applications to the quantitative determination of virtually all subtypes of the influenza A viral hemagglutinins. Vaccine 26 (48), 6068–6076.
Colman, P.M., 1992. Structural basis of antigenic variation: studies of influenza virus neuraminidase. Immunol. Cell Biol 70 (Pt 3), 209–214.
Damian, C.E., Ian, A., 2012. Wilson Broadly neutralizing antibodies against influenza virus and prospects for universal therapies. Curr. Opin. Virol. 2 (2), 134–141.
Dotis, J., Roilides, E., 2009. H1N1 Influenza A infection. Hippokratia 13 (3), 135–138.
Doytchinova, I.A., Flower, D.R., 2007. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinform. 5, 8–14.
Ekiert, D.C., Bhabha, G., Elsliger, M.A., Friesen, R.H.E., Jongeneelen, M., Throsby, M., Goudsmit, J., Wilson, I.A., 2009. Antibody recognition of a highly conserved influenza virus epitope. Science 324 (5924), 246–251.
El-Manzalawy, Y., Honavar, V., 2010. Recent advances in B-cell epitope prediction methods. Immunome. Res. 3:6 **2**(Suppl 2):S2.
Gao R, Cao B, Hu Y et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med 2013,368(20):1888-97.
Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Raghava, G.P.S., 2013. In silico approach for predicting toxicity of peptides and proteins. PLoS One 2013; 8(9):e73957.
Hua, C.K., Gacerez, A.T., Sentman, C.L., Ackerman, M.E., Choi, Y., Kellogg, C.B., 2017. Computationally driven identification of antibody epitopes. Elife 2017 (4), 6–e29023.
Huang, H.W., Honda, W., 2006. CED: a conformational epitope database. BMC. Immunol. 7, 7.
Hui, D.S.C., Lee, N., Chan, P.K.S., 2017. A clinical approach to the threat of emerging influenza viruses in the Asia-Pacific region. Respirology 22 (7), 1300–1312.
Iurescia, S., Fioretti, D., Fazio, V.M., Rinaldi, M., 2012. Epitope-driven DNA vaccine design employing immunoinformatics against B-cell lymphoma: a biotech's challenge. Biotechnol. Adv. 30 (1), 372–383.
Jazayeri, S.D., Poh, C.L., 2019. Development of Universal Influenza Vaccines Targeting Conserved Viral Proteins. Vaccines 7 (4), 169.
Jeffery, K.T., David, M.M., 2010. Influenza: The Once and Future Pandemic. Public Health Rep. 25 (Suppl 3), 16–26.
Karplus, P.A., Schultz, G., 1985. Prediction of chain flexibility in proteins. Naturwissenschaften. 72, 212–213.
Katherine, H., Kanta, S., 2015. Influenza Vaccines: Challenges and Solutions. Cell Host Microbe. 17 (3), 295–300.
Khan, M.A., Hossain, M.U., Rakib, S.M., Moorshed, M.M., 2015. Epitope-based peptide vaccine design and target site depiction against Ebola viruses: an immunoinformatics study. Scand. J. Immunol. 82 (1), 25–34.
Kolaskar, A., Tongaonkar, P., 1990. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett. 276 (1–2), 172–174.
Kumar, A., Meldgaard, T.S., Bertholet, S., 2018. Novel Platforms for the Development of a Universal Influenza Vaccine. Front Immunol. 23, 9–60.
Lenka, P., Mangesh, B., Lucia, B.P., 2016. An Introduction to B-Cell Epitope Mapping and In Silico Epitope Prediction. Immunol. Res. 2016, 6760830.
Li, W., Cowley, A., Uludag, M., Gur, T., McWilliam, H., Squizzato, S., Park, Y.M., Buso, N., Lopez, R., 2015. The EMBL-EBI bioinformatics web and programmatic tools framework. Nucleic Acids Res. 43 (W1), W580–W584.
Lisa, R.C., 2011. Influenza Update. A Review of Currently Available Vaccines. Pharm. Therap. 36 (10), 659–684.
Lohia, N., Baranwal, M., 2015. Identification of Conserved Peptides Comprising Multiple T Cell Epitopes of Matrix 1 Protein in H1N1 Influenza Virus. Viral. Immunol. 28 (10), 570–579.
Lu, Y., Ding, J., Liu, W., Chen, 2002. Y.H., A candidate vaccine against influenza virus intensively improved the immunogenicity of a neutralizing epitope. Int. Arch. Allergy Immunol.127(3):245–250.
Maleki, A., Russo, G., Palumbo, G.A.P., Pappalardo, F., 2022. In silico design of recombinant multi-epitope vaccine against influenza A virus. BMC Bioinform. 2;22 (Suppl 14), 617.
Monto, A.S., 1987. Influenza: quantifying morbidity and mortality. Am. J. Med. 82, 20–25.
Noronha, J.M., Liu, M., Squires, R.B., Pickett, B.E., Hale, B.G., Air, G.M., Galloway, S.E., Toru Takimoto, Mirco Schmolke, M., Hunt, V., Klem, E., Sastre, A.G., McGee, Scheuermann, R.H., 2012. Influenza virus sequence feature variant type analysis: evidence of role for NS1 in influenza virus host range restriction. J. Virol. 86(10):5857–5866.
Oostenbrink, C., Gunsteren, V.W.F., 2004. Free energies of binding of polychlorinated biphenyls to the estrogen receptor from a single simulation. Proteins 2004; 54(2):237–246.
Palese, P., Tobita, K., Ueda, M., 1974. Characterization of temperature sensitive influenza virus mutants defective in neuraminidase. Virology 61 (2), 397–410.
Parker, J.M., Guo, D., Hodges, R.S., 1986. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry 25 (19), 5425–5432.
Patronov, A., Doytchinova, I., 2013. T-cell epitope vaccine design by immunoinformatics. Open Biol. 3, (1) 120139.
Peterson JM, O'Leary CA, Moss WN., 2022. In silico analysis of local RNA secondary structure in influenza virus A, B and C finds evidence of widespread ordered stability but little evidence of significant covariation. Sci. Rep. 2022 Jan 10;12 (1):310.
Petrosillo, N., Di Bella, S., Drapeau, C.M., Grilli, E., 2009. The novel influenza A (H1N1) virus pandemic: An update. Ann. Thorac. Med. 4 (4), 163–172.
Saha, S., Raghava, G.P.S., 2006. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins Struct. Function Bioinform. 65 (1), 40–48.

Sakib, M.S., Islam, M.R., Hasan, A.K., NabI, A.H.M.N., 2014. Prediction of epitope-based peptides for the utility of vaccine development from fusion and glycoprotein of nipah virus using in silico approach. Adv. Bioinform. 2014, 402492.
Sathish, S., Mageshbabu, R., Subramanian, S., Nandagopal, B., Sridharan, G., 2018. Design of peptide epitope from the neuraminidase protein of influenza A and influenza B towards short peptide vaccine development. Bioinformation 14 (5), 183–189.
Skehel, J.J., Wiley, D.C., 2000. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu. Rev. Biochem. 69, 531–569.
Smet, A., Catani, J.P.P., Ysenbaert, T., Gonçalves, A., Kleanthous, H., Vogel, T.U., Saelens, X., Job, E.R., 2022. Antibodies directed towards neuraminidase restrict influenza virus replication in primary human bronchial epithelial cells. PLoS One 17, (1) e0262873.
Sun, P., Ju, H., Liu, Z., Ning, Q., Zhang, J., Zhao, X., Huang, Y., Ma, Z., Li, Y., 2013. Bioinformatics resources and tools for conformational B-cell epitope prediction. Comput. Math Methods Med. 2013, 943636.
Tan, P.T., Heiny, A.T., Miotto, O., Salmon, J., Marques, E.T.A., Lemonnier, F., Thomas, J., 2010. Conservation and diversity of influenza A H1N1 HLA-restricted T cell epitope candidates for epitope-based vaccines. PLoS One 2010; 5(1):e8754.
Testa, J.S., Philip, R., 2012. Role of T-cell epitope-based vaccine in prophylactic and therapeutic applications. Future Virol. 7 (11), 1077–1088.
Thundimadathil, J., 2012. Cancer treatment using peptides: current therapies and prospects. J. Amino Acids 2012, 967347.
Tregoning, J.S., Russell, R.F., Kinnear, E., 2018. Adjuvanted influenza vaccines. Hum. Vaccin. Immunother. 14 (3), 550–564.
Trowsdale, J., Knight, J.C., 2013. Major histocompatibility complex genomics and human disease. Annu. Rev. Genomics Hum. Genet. 14, 301–323.
Utpal, K.A., Mourad, T., Mizanur, R., 2018. Immunoinformatics Approach for Epitope-Based Peptide Vaccine Design and Active Site Prediction against Polyprotein of Emerging Oropouche Virus. J. Immunol. Res., 2018–6718083
Vita, R., Zarebski, L., Greenbaum, J.A., Emami, H., Hoof, I., Salimi, N., Damle, R., Sette, A., Peters, B., 2009. The Immune Epitope Database 2.0. Nucleic Acids Res. 38 (Database issue), D854–D862.
Ward, F., Sinu, P., Sandeep, K.D., Mahajan, S., Xu, X., Peters, B., Sette, A., 2017. The Immune Epitope Database and Analysis Resource in Epitope Discovery and Synthetic Vaccine Design. Front. Immunol. 2017 (14), 8–278.
Wang, C., Greene, D.A., Xiao, L., Qi, R., Luo, R., 2018. Recent Developments and Applications of the MMPBSA Method. Front Mol Biosci 10 (4), 87.
Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2–a multiple sequence alignment editor and analysis workbench. Bioinformatics 25 (9), 1189–1191.
Zheng, J., Lin, X., Wang, X., Zheng, L., Lan, S., Jin, S., Ou, Z., Wu, J., 2017. In Silico Analysis of Epitope-Based Vaccine Candidates against Hepatitis B Virus Polymerase Protein. Viruses 9 (5), 112.
Zhu, S., Xue, X., Liu, J., Zhao, P., Wang, J., Li, W., Zhang, L., 2010. Expression of HPV6b L1/EBV LMP2 multiepitope and immunogenicity in mice. Acta Biochim. Biophys. Sin 42 (8), 515–521.

Further reading

Peters, B., Bui, H.H., Frankild, S., Nielson, S.F.M., Lundegaard, C., Kostem, E., Basch, D., Lamberth, K., Harndahl, M., Fleri, W., Wilson, S.S., Sidney, J., Lund, O., Buus, S., Sette, A., 2006. A community resource benchmarking predictions of peptide binding to MHC-I molecules. PLoS Comput. Biol. 2, (6) e65.