ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 01 | January - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EPITOPE PREDICTION USING BIOINFORMATICS AND ITS IMPLICATION IN VACCINE DESIGN

Immunology

Anu John B* Graden city University, Bangalore. *Corresponding Author

Dr. L.A.Rama Chandra Prasad Graden city University, Bangalore.

ABSTRACT

Traditional method of vaccine production comprises of live or fixed whole pathogen used in vaccine productions or protein antigens which are purified for vaccine development or vaccine that comprises of DNA plasmids that measures the expression of sequence of the most important pathogen protein antigens in the host. However traditional method of vaccine development are tedious process and Loss of efficacy is noted.
A new evolution in vaccine development replacing the traditional method has been developed using the approaches of Bioinformatics tools known as the epitope prediction. Epitopes are short amino acid sequences of a protein that are capable of inducing a more direct and potent immune response, than the response induced by the whole cognate protein. Mapping of B cell and T cell epitope is crucial step in developing epitope-based vaccine, therapeutic antibody. Several bioinformatic tools are employed to predict the B- cell and T- cell Epitopes based on the sequence or structural data. The fundamental objective of epitope prediction is replacing the antigen in immunization. This paper comprises of the experimental methods on how to predict an epitope using bioinformatic approaches and in silico resources.

KEYWORDS

Epitope Prediction, Immunoinformatics, Vaccine development, Insilico approaches

INTRODUCTION

Exploring Vaccine designing tools has helped in combating against pathogenic or Infectious diseases like Influenza, smallpox, chikungunya, polio, tetanus, and many other deadly diseases. Conventional method of vaccine development using live or attenuated pathogens is a tedious process and might take 10-15 years of development, which includes Selection of strains for vaccine production, Growing the organism , Isolating and purification of the organism, Inactivation of the vaccine, Formulation of vaccine and Quality Control. Although these vaccines could save lives but can be unfavorable due to cause of Adverse events and might even lead to death.

Immunoinformatic has paved a way to the development of vaccines using epitope prediction tools using computational technology which can store and organize large amount of biological data generated from genetics, molecular biology, and Biotechnology. Immunoinformatics is a science that understands the immunological information through computational techniques. Immunoinformatic tools deals with huge amount of data related to Immune. Shortening of the time required to conduct the experiment is another benefit of using computational methods.

Antigen and antibody interaction are a major event in humoral immune response that helps invading pathogen. A specific antibody (Ab) recognizes antigen (Ag) at regions known as antigenic determinants or B-cell epitopes. B-cell epitopes is a surface accessible clusters of amino acids, which are recognized by secreted antibodies or B-cell receptors and can elicit cellular or humoral immune response. B-cells and T-Cells play a major role in understanding the antigenic interactions, and are also implicated in vaccine designs, prevention of disease, diagnostics and therapeutically valuable. (Yang and Yu, 2009).) Identification of a correct epitope impacts the molecular effect of antibodies interaction with the antibodies. There are several existing methods for identifying an B -cell or T-cell epitope that includes, X-Ray crystallography, pepscan, phage display, expressed fragments, partial proteolysis, mass spectrometry, and mutagenesis analysis (Xu et al., 2010). However, these methods are not only expensive but time consuming and fail to identify many epitopes (Inbal Sela-Culang, *et al, 2014*) and more over these methods just identify the linear stretches as epitopes which might be discontinuous. Even while X-ray crystallography is maybe the most reliable technique for epitope identification (Sun et al., 2011), it is expensive, time consuming, and very difficult to apply for many targets.

To overcome all these drawbacks development of bioinformatics tools are very essential, advances in recombinant DNA technology (rDNA) and the knowledge on the host immune system and the genetic information of the pathogen will pave the way to design a new vaccine against diseases that currently has few or no measures in controlling the disease within a few span of years ie 1 or 2 years using computational biology and in silico predictions to define the target (Jack wood, et al.,2008) . The vaccines developed through rDNA technologies are designed to be safer, more efficacious, and or less expensive than conventional vaccines. To achieve these aims, a thorough understanding of the disease agent, particularly, critical epitopes to induce the appropriate immunological reaction is required.

The major step in designing a multi-epitope vaccine involves the retrieval of the genome sequence and identifying the protein component of the pathogen that could elicit an immune response. The strategy of designing the vaccine requires the completed knowledge of the sequence of the pathogenic protein. Reverse vaccinology is used to elicit an immune response against the pathogen using the information of the codon sequence present in the DNA of the pathogen to obtain a complementary cDNA, and further translating it to obtain the sequence of the protein of interest. Once the Protein of interest is generated inside the Antigen presenting cells (APC) of the host. The T cell epitopes are then proteolytically cleaved from the protein, and further exposed by the MHC molecules of the APC surface, to interact with the receptors of T cells. Therefore, with the knowledge of the primary sequence of the protein antigen, the epitopes can be identified by cloning the domains or smaller peptides of the protein separately and experimentally determining which one is more immunogenic, or alternatively, by screening the whole protein sequence using in silico predictions programs (Fleri et al.;2017).

These computational techniques help in accurate diagnosis of disease and producing cost effective vaccines, developing more effective and new drugs and helps in improving the quality and quantity of the therapeutic product and also helps in understanding and discovering immunological issues.

METHODOLOGY

Sequence Retrieval:

The first step in vaccine designing in the sequence retrieval of the protein component of the pathogen. The NCBI is the most highly referred biological sequence source. It contains huge records of RNA, DNA (including genomic DNA), and protein sequences [5]. A comprehensive amount of information about the protein sequences and their annotation is provided in the Universal Protein Resource (UniProt) at (https://www.uniprot. org/). The UniProt databases include UniProt Knowledgebase (UniProtKB), UniProt Archive (UniParc), and UniProt Reference Clusters (UniRef) [6]. Protein sequences are stored in these databases as records containing various cellular, molecular, biological, functional, and structural information along with external links for further information .RCSB PDB (Protein data bank) is also one of the highly referred databases containing data about the 3D structures of proteins, nucleic acids, and their complexes [7]. The information obtained from this database helps the researchers to get a better grasp about the various aspects of biomedicine and

agriculture [8]. Innate DB (http://www.innatedb.ca) is a database dedicated to facilitating systems level investigations of innate immune response for the mammalian (human, mouse and bovine).It has been developed to bring about more sophisticated perception about the innate immune system responses. To date, more than 27,172 curated interactions, 5237 reviewed publications, and 9460 curated genes are deposited in InnateDB. Cerebral is the newer version of InnateDB [9]. The scientific databases and software tools could also be accessed by ExPASy which is the SIB Bioinformatics Resource Portal at (https://www.expasy.org/). The ExPASy portal gives the scientists the convenient access to various data including genomics, transcriptomics, proteomics, phylogeny, population genetics, systems biology etc. A large amount of sequential and structural data about numerous proteins is currently stored owing to rapidly growing number of unveiled genomic sequences and high throughput resolving of protein structures. These data could effectively be used to extract Functional insight for medical and biological purposes. Molecular dynamics simulations and bioinformatics predictions are the two main computational techniques which could be exploited to reach this goal. With the sequence provided, bioinformatics tools can be used to employ statistical and structural analyses to understand their function, annotate the genome and predict the protein structures. Bioinformatics method is one of the most useful technologies used in science which is used to study huge amount of data. Also, to invent new insights about theories and protein design, using algorithms of sequence and structure alignment. [10]

Epitope Prediction

B-Cell Epitope

Designing of B-cell epitope is one of the crucial steps in vaccine designing. immunodiagnostic design and immunogen design for antibody production efforts. B-cell epitopes are determinant regions of the antigen with the ability to bind immunoglobulins and B-cell receptors (BCRs). The binding site of BCR is hydrophobic, contains six hypervariable loops which are variable regarding their amino acid composition and length. Since conventional methods are time consuming and a tedious process, computational methods are employed in predicting the B-cell epitope. Since computational methods are more reliable and an alternative source for identification of putative B-cell epitopes. B-cell epitopes are classified into linear (continuous) and conformational (discontinuous).

Linear epitopes are short peptides that are composed of sequential amino acids in the antigen. However, most B-cell epitopes are found to be discontinuous (90% of B-cell epitopes), where the amino acids of epitope may not be contiguous in the primary sequence and spatial proximity is attained by protein folding. Mapping of B-Cell epitope is the most important aspect in experimental and computational epitope prediction. There are both sequence-based and structure-based prediction tools are available however only limited number of tools are used in discontinuous B-cell epitopes prediction.

Discontinuous B-cell epitopes comprises of peptides with linear amino acid. Identification of B-cell epitopes can invoke strong immune responses which plays a vital role in effective vaccine designing. Sequence-based methods, machine-learning methods, and amino acid propensity scale-based methods are the main methodologies used in continuous B-Cell Epitope Prediction [11].

Sequence-based methods generally work on the bases of surface accessibility of epitopes for antibody binding [12]. However, one of the drawbacks of using this method is that these methods are only limited to the prediction of continuous epitopes.

Amino Acid Propensity methods are based on Scale that correlates the location of continuous epitopes with analyzing parameters such as accessibility, hydrophilicity, flexibility, exposed surface, polarity turns, and antigenic propensity of polypeptide chains. Thus, the prediction of linear B-cell epitopes typically relies on the use of amino acid propensity scales [13]. Amino acid scales are applied to compute the scores of residues in amino acid scale-based methods. Approximately 60-66% of accuracy are obtained in epitope prediction using Amino acid-based method. By improving the quality of the existing B-Cell epitope datasets a high accuracy of epitope design can be obtained. There are several tools that can be used in B-Cell Epitope prediction, among which includes the Bcepred tool which predict the linear B-Cell epitope using physicochemical properties such as hydrophilicity, flexibility, polarity, and exposed surface on a non-redundant dataset. The dataset contains 1029 B-cell epitopes obtained from Bcipep database and an equal number of non-epitopes obtained randomly from Swiss-Prot database. The prediction accuracy for models based on these properties varies from 52.92% to 57.53% [14]. The ABCpred server, which is also a widely used tools used to predict the B-cell Epitope, which is based on neural networks, has an estimatedaccuracy of 65.93% [15]. Another server called BepiPred predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method. Another major tool used in epitope prediction is Disco Tope which uses the three-dimensional structure of proteins to determinate the surface accessibility and a novel epitope propensity amino acid score. The final scores are calculated by combining the propensity scores of residues in spatial proximity and the contact numbers. This server is also used to predicts epitopes in complexes of multiple chains [16]. This tool along with BEpro (formerly known as PEPITO) and SEPPA (Spatial Epitope Prediction of Protein Antigens) requires a 3-D structure as input, specifically, in PDB format. Using SEPPA, each residue in the query protein will be given a score according to information from its neighborhood residues. The higher score corresponds to the higher probability of the residue to be involved in an epitope. One of the most reliable tools in epitope prediction is the ElliPro This server is used to predicts linear and discontinuous epitopes based on a protein antigen's 3-D structure. ElliPro associates each predicted epitope with a score, defined as a PI (Protrusion Index) value. Comparatively with the databases mentioned earlier, in ElliPro the input is a protein sequence. A 3-D structure will be predicted for the input protein sequence by homology modeling based on user-selected structural template. Later linear and discontinuous epitopes will be computed based on the predicted protein structure. Also, the prediction task is more difficult compared to that of T-cell epitopes. Changes in protein folding and 3D structure may lead to changes in the number of epitopes. Although significant breakthroughs have been made in the field of B-cell epitope prediction X-ray crystallography continues to be the most accurate way to identify B-cell epitope.Few other bioinformatics tools that are used to predict continuous and discontinuous B cell epitopes are included in Table 1.

All these integrative tools represent an opportunity for the development of new vaccines in special those that aim at the elicitation of humoral responses.

Table 1: A List Of Reliable Tools And Databases For B-cell Epitope Prediction.

Database name	Web address	Application	Ref
ABCpred	http://www.imtech.res.in/raghava/abcpred	Prediction of linear B-cell epitopes based on artificial neural net	[17]
BCIPEP	http://www.imtech.res.in/raghava/bcipep	B-cell epitope database	[18]
Bepipred	http://www.cbs.dtu.dk/services/BepiPred	Prediction of B-cell epitopes using HMM method	[19]
IMGT	http://www.imgt.org	A high-quality integrated resource of IG, TR, MHC, and related proteins	[17]
Bcepred	http://www.imtech.res.in/raghava/bcepred/	Prediction of epitopes with 58.7% accuracy	[20]
BEPITOPE	jlpellequer@cea.fr	Prediction of linear epitopes location and pattern	[21]
DiscoTope	http://www.cbs.dtu.dk/services/DiscoTope/	Prediction of conformational B-cell epitopes based on 3D structure	[22]
COBEpro	http://scratch.proteomics.ics.uci.edu	A two-stage system to predict linear B-cell epitopes, associated with the SCRATCH database	[23]
CEP	http://bioinfo.ernet.in/cep.htm	Prediction of B-cell epitopes	[24]
AgAbDb	http://www.115.111.37.206:8080/agabdb2/home.jsp	Antigen-antibody interaction database	[25]

MIMOP	Request from franck.molina@cpbs.univ-montp1.fr	prediction of 3D epitopic region from the mimotope peptide sequences	[26]
MIMOX	http://www.immunet.cn/mimox/	Epitope mapping based on phage display method	[27]
Pepitope	http://www.pepitope.tau.ac.il/	Epitope mapping based on affinity-selected peptides	[28]
3DEX	http://www.schreiber-abc.com/3dex/	Conformational epitopes mapping in 3D protein structures	[29]
IEDB	http://www.immuneepitope.org	Epitope prediction database	[30]
AntiJen	http://www.jenner.ac.uk/antijen/	Quantitative binding data for B-cell epitopes	[31]
CED	http://immunet.cn/ced	Conformational Epitope Database	[32]

T-Cell Epitope

One of the most important steps to develop an epitope-based vaccine, is the selection of T-cell epitopes with strong binding affinity to appropriate HLA molecules which are mainly predicted by matrix-based method [32]. Accurate prediction of interacting molecules is the current challenge in immunological prediction of T-cell epitope sequences. At present binding affinity predictions for a range of MHC molecules is considered to be the most popular epitope prediction methods. For recognition of cytotoxic T-cells Molecular binding between antigenic peptides and MHC molecules is necessary. Therefore, for any T-cell epitopes prediction algorithms need to identify MHC-binding peptides.Peptide binding to MHC and the interaction of this complex with a specific T-cell receptor are the requirements for a functional T-cell response. A Well-defined data is required for modeling the peptides that binds to TAP and MHCs. Epitope mapping could lead to design of effective peptide vaccines [33]. The ability of the peptide immunogens to induce CTL activity can be enhanced by inclusion of T-helper epitopic peptides. Among the Prediction tools "PADRE (PanDR)" is one of the universal T-cell helper epitopes which could be used as a general tool to enhance CTL responses of different antigens [35]. Different methodologies are used to predict peptides which has the ability to bind MHC molecules these includes Hidden Markov model (HMM), Artificial neural networks (ANNs), Support Vector Machine (SVM), and Quantitative matrices [36]. Various databases have been experimentally determined T-cell epitopes and different tools have been created for T-cell epitopes prediction. Among the numerous servers for T-Cell Prediction is the RANKPEP, which predicts peptide binders to MHC-I and MHC-II molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). It is also used to predict the MHC-I ligands whose C-terminal end is likely to be the result of proteasomal cleavage. It is one of the friendly platforms which offers the widest allelic coverage to MHC-I and MHC-II alleles (118 and 67 alleles, respectively) for human and mouse. To search epitopes sequences for MHC-I ligands using PSSMs, a dynamic algorithm written in Python is used, which scores all protein segments with the length of the PSSM width and sorted accordingly. Scoring starts at the beginning of each sequence and the PSSM is slid over the sequence one residue at a time until reaching the end of the sequence. This binding threshold is built into each matrix, delineating the range of putative binders among the top scoring peptides. The IEDB Analysis Resource database uses NetMHCpan which is used as a prediction method since 2011. This method generates a quantitative prediction of the affinity of any peptide-MHC class I interaction, covering HLA-A and HLA-B. nHLAPred is another comprehensive tool for the prediction of MHCI binding peptides for 67 MHC alleles. Artificial Neural Networks (ANNs) and quantitative matrices (QM is used in the prediction of alleles. The predicted MHC binders are filtered to potential CTL epitopes by using proteasomal matrices. These servers have two options (Compred and ANNPred), the most wide-ranging is Compred; based on the hybrid approach of artificial neural networks and quantitative matrices NetMHC server is used to predict binding of peptides to a number of different HLA alleles using ANNs. ANNs have been trained for 78 different human MHC (HLA) alleles representing all 12 HLA-A and -B Supertypes. ProPred1 is one of the server that predicts MHC Class-II binding regions in an antigen sequence using quantitative matrices. The server is employed in Assisting of locating promiscuous binding regions that are useful in selecting vaccine candidates covering 51 alleles [37]. The SVMHC server enables prediction of both MHC class I and MHC class II binding peptides, however, it is most widely covered is for MHC-II (51 alleles). The graphical output displayed in this software also allows for simple identification of promiscuous epitopes. SVMHC uses the matrices developed by the TEPITOPE software. A list of reliable tools and databases for T-cell epitope prediction is listed below in Table 2.

Table 2: A List Of Reliable Tools And Databases For T-cell Epitope Prediction

Database name	Web address	Application	Ref
Allele frequencies	http://www.allelefrequencies.net	HLA frequencies in worldwide population and polymorphism frequencies in immunologically	[38]
MHCPred	http://www.ddg-pharmfac.net/mhcpred/MHCPred/	Quantitative prediction of peptide-MHC binding	[39]
MMBPred	http://www.imtech.res.in/raghava/mmbpred/	Prediction of atypical MHC class I binders as well as mutations that allow high-affinity binding	[40]
NetCTL	http://www.cbs.dtu.dk/services/NetCTL	Prediction of CTL/HLA supertype epitopes	[41]
NetMHC	http://www.cbs.dtu.dk/services/NetMHC	Prediction of the MHC binding propensity of peptides	[42]
NetChop	http://www.cbs.dtu.dk/services/NetChop	Prediction of proteasome/immunoproteasome cleavage	[43]
TAPPred	http://www.imtech.res.in/raghava/tappred/	Prediction of binding affinity of TAP protein	[44]
Pcleavage	http://www.imtech.res.in/raghava/pcleavage/	Prediction of proteasome/immunoproteasome cleavage	[45]
Propred	http://www.imtech.res.in/raghava/propred1/	Prediction of class II binding regions in antigenic protein sequence	[46]
ElliPro	http://www.tools.immuneepitope.org/tools/ElliPro	Prediction of linear and conformational antibody epitopes	[47]
EpiToolKit	http://www.epitoolkit.org	Prediction of MHC classes I/II ligands using several methods	[48]
PREDEPP	http://margalit.huji.ac.il/Teppred/mhc-bind/index.html	Prediction of MHC-peptide binding based on structure	[49]
PDB	https://www.rcsb.org/pdb/https://www.rcsb.org/pdb/	MHC/peptide/TCR combinations	[50]
TEPITOPE	https://www.vaccinome.com	Prediction of atypical class II epitopes	[51]

Summary

Immunoinformatics in recent era has played a crucial role in improving the immunological studies. The major focus on B- and T-cell epitopes prediction methods have become crucial step in immunoinformatics studies due to their efficiency in applications like epitope-based design of personalized cancer immunotherapies and therapeutic/ prophylactic vaccines. The high throughput development methods have brought about novel immunological knowledge as well as new challenges. Predictions for class epitopes have reached a Peak point where only little improvements are required to obtain a possible results of HLA binding assays with the help of high-throughput approaches. Moreover, huge amount of data is obtained and can be easily analyzed with the help of Immunoinformatics. Through the study of genomes and their variations a huge amount of data is obtained which has opened a wide horizon in the field of rational design of prophylactic vaccines .These vaccines can be easily designed using vaccine design tool and highly accurate methods of T-cell epitope prediction. Drastic Increase in disease count has made it difficult for the production of vaccines by conventional method, however these drawbacks could be ruled out with the help of Drug designing using computational biology. Genomic sequencing of cancer patients would provide novel options to design personalized immunotherapies and chemotherapeutic remedies. Although the options to design personalized immunotherapies, remain to be experimental, they have garnered a lot of interest. A new evolution in vaccine development replacing the traditional method has been developed using the approaches of

Bioinformatics tools known as the epitope prediction. Epitopes are short amino acid sequences of a protein that are capable of inducing a more direct and potent immune response, than the response induced by the whole cognate protein. Mapping of B cell and T cell epitope is crucial step in developing epitope-based vaccine, therapeutic antibody. Several bioinformatic tools are employed to predict the B-cell and T- cell Epitopes based on the sequence or structural data.

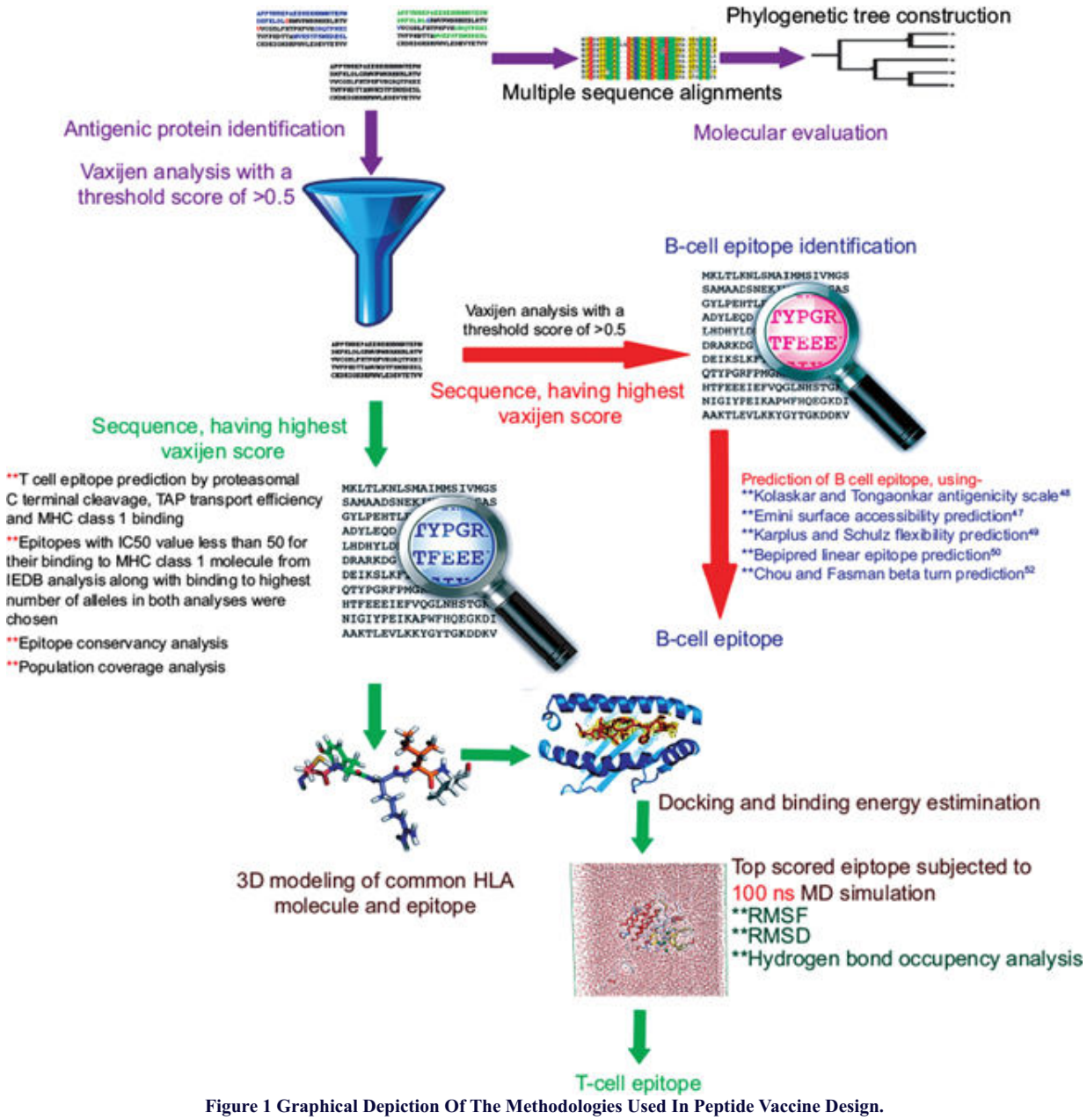

Figure 1 Graphical Depiction Of The Methodologies Used In Peptide Vaccine Design.

Conflicts Of Interest:
The authors declare no conflict of interest.

Compliance With Ethical Statement:
Since this is a review article ethical approval is not applicable.

Informed Consent:
The Authors confirmed that authorized informed Was obtained.

Ethical Approval:
Since this is a review article ethical approval is not applicable.

REFERENCES

1. Jackwood MW, Hickle L, Kapil S, Silva R. Vaccine development using recombinant DNA technology. Council Agric Sci Technol 2008;38:1–11.
2. Jurtz V, Paul S, Andreatta M, Marcatili P, Peters B, Nielsen M. NetMHCpan- 4.0: improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. J Immunol (2017) 199:3360–8. doi:10.4049/jimmunol.1700893
3. Consortium U. UniProt: the universal protein knowledgebase. Nucleic Acids Res 2018;46(5):2699.
4. Van Regenmortel MHV. Synthetic peptide vaccines and the search for neutralization B cell epitopes. Open Vaccine J (2009) 2:33–44. doi:10.2174/ 1875035401002010033
5. Coordinators NR. Database resources of the national center for biotechnology information. Nucleic Acids Res 2016;44(Database issue):D7.
6. Coordinators NR. Database resources of the national center for biotechnology information. Nucleic Acids Res 2018;46(Database issue):D8.
7. Consortium U. UniProt: the universal protein knowledgebase. NucelicAids Res2018;46(5):2699.
8. Kleywegt GJ, Velankar S, Patwardhan A. Structural biology data archiving: where we are and what lies ahead. FEBS Lett 2018;592:2153. doi:10.1002/1873-3468.13086.
9. Lynn DJ, Winsor GL, Chan C, et al. InnateDB: facilitating systems-level analyses of the mammalian innate immune response. Mol Syst Biol 2008;4(1): 218. doi:10.1038/msb.2008.55.
10. Moult J, Fidelis K, Kryshtafovych A, et al. Critical assessment of methods of protein structure prediction: progress and new directions in round XI. Proteins Struct Funct Bioinform 2016;84:4–14. doi:10.1002/prot.25064.
11. Ramanathan B, Poh CL, Kirk K, et al. Synthetic BCell epitopes eliciting cross-neutralizing antibodies: strategies for future dengue vaccine. PLoS One 2016;11(5): e0155900. doi:10.1371/journal.pone.0155900.
12. Yang X, Yu X. An introduction to epitope prediction methods and software. Rev Med Virol 2009;19(2):77–96. doi:10.1002/rmv.602.
13. Chen J, Liu H, Yang J, et al. Prediction of linear Bcell epitopes using amino acid pair antigenicity scale. Amino Acids 2007;33(3):423–428. doi:10.1007/ s00726-006-0485-9.
14. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins 2006;65:40–8.
15. Sun P, Ju H, Liu Z, Ning Q, Zhang J, Zhao X, et al. Bioinformatics resources and tools for conformational B-cell epitope prediction. Comput Math Methods Med

2013;2013:943636.
16. Kringelum JV, Lundegaard C, Lund O, Nielsen M. Reliable B cell epitope predictions: impacts of method development and improved benchmarking. Plos Comput Biol 2012;8:12.
17. Sun P, Ju H, Liu Z, et al. Bioinformatics resources and tools for conformational B-cell epitope prediction. Comput Math Methods Med 2013;2013:1.doi:10.1155/2013/943636.
18. Saha S, Bhasin M, Raghava GP. Bcipep: a database of B-cell epitopes. BMC Genomics 2005;6(1):79. doi:10.1186/1471-2164-6-79.
19. Larsen JEP, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. Immunome Res 2006;2(1):2. doi:10.1186/1745-7580-2-2.
20. Saha S, Raghava G. BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties. Paper Presented at:International Conference on Artificial ImmuneSystems. 2004Odorico M, Pellequer JL. BEPITOPE: predicting thelocation of continuous epitopes and patterns in proteins.J Mol Recogn 2003;16(1):20–22. doi:10.1002/jmr.602.
21. Haste Andersen P, Nielsen M, Lund O. Prediction ofresidues in discontinuous B-cell epitopes using protein 3D structures. Protein Sci 2006;15(11):2558–2567. doi:10.1110/ps.062405906.
22. Sweredoski MJ, Baldi P. COBEpro: a novel system for predicting continuous B-cell epitopes. Protein Eng Design Select 2008;22(3):113–120. doi:10.1093/ protein/gzn075.
23. Kulkarni-Kale U, Bhosle S, Kolaskar AS. CEP: a conformational epitope prediction server. Nucleic Acids Res 2005;33(suppl_2):W168–W171. doi:10.1093/nar/ gki460.
24. Ghate A, Bhagwat B, Bhosle S, et al. Characterization of antibody-binding sites on proteins: development of a knowledgebase and its applications in improving epitope prediction. Protein Peptide Lett 2007;14(6):531–535.
25. Moreau V, Granier C, Villard S, et al. Discontinuous epitope prediction based on mimotope analysis. INTERNATIONAL REVIEWS OF IMMUNOLOGY 15 Bioinformatics 2006;22(9):1088–1095. doi:10.1093/bioinformatics/btl012.
26. Huang J, Gutteridge A, Honda W, et al. MIMOX: a web tool for phage display based epitope mapping.BMC Bioinform 2006;7(1):451 .doi:10.1186/1471-2105-7-451.
27. Mayrose I, Penn O, Erez E, et al. Pepitope: epitope mapping from affinity-selected peptides.Bioinformatics 2007;23(23):3244–3246. doi:10.1093/ bioinformatics/btm493.
28. Schreiber A, Humbert M, Benz A, et al. 3D-Epitope- Explorer (3DEX): Localization of conformational epitopes within three-dimensional structures of proteins.J Computer Chem 2005;26(9):879–887. doi:10. 1002/jcc.20229.
29. Toseland CP, Clayton DJ, McSparron H, et al.AntiJen: a quantitative immunology database integrating functional, thermodynamic, kinetic, biophysical, and cellular data. Immunome Res 2005;1(1):4.
30. Huang J, Honda W. CED: a conformational epitope database. BMC Immunol 2006;7(1):7. doi:10.1186/ 1471-2172-7-7.
31. Chen X, Zaro JL, Shen W-C. Fusion protein linkers:property, design and functionality. Adv Drug Deliv Rev 2013;65(10):1357–1369. doi:10.1016/j.addr.2012.09.039.
32. Russell-Jones GJ, Geczy AF. T-cell epitopes In:Google patents; 1999.
33. Patronov A, Doytchinova I. T-cell epitope vaccine design by immunoinformatics. Open Biol 2013;3(1): 120–139. doi:10.1098/rsob.120139.
34. Tomar N, De RK. Immunoinformatics: an integratedscenario. Immunology 2010;131(2):153–168. doi:10.1111/j.1365-2567.2010.03330.x.
35. Engler OB, Dai WJ, Sette A, et al. Peptide vaccines against hepatitis B virus: from animal model to human studies. Mol Immunol 2001;38(6):457–465.
36. Flower DR. Towards in silico prediction of immunogenic epitopes. Trends Immunol 2003;24(12): 667–674
37. Singh H, Raghava GPS. ProPred: Prediction of HLA-DR binding sites. Bioinformatics 2001;17:1236–7.
38. Gonzalez-Galarza FF, Christmas S, Middleton D,et al. Allele frequency net: a database and online repository for immune gene frequencies in worldwide populations. Nucleic Acids Res 2011;39 (suppl_1):D913–D919. doi:10.1093/nar/gkq1128.
39. Guan P, Doytchinova IA, Zygouri C, et al. MHCPred: a server for quantitative prediction of peptide–MHC binding. Nucleic Acids Res 2003; 31(13):3621–3624. doi:10.1093/nar/gkg510.
40. Bhasin M, Raghava G. Prediction of promiscuous and high-affinity mutated MHC binders. Hybrid Hybridom 2003;22(4):229–234. doi:10.1089/ 153685903322328956.
41. Heiny A, Miotto O, Srinivasan KN, et al. Evolutionarily conserved protein sequences of influenza a viruses, avian and human, as vaccine targets. PLoS One 2007;2(11):e1190. doi:10.1371/journal. pone.0001190.
42. Lundegaard C, Lamberth K, Harndahl M, et al. NetMHC-3.0: accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8–11. Nucleic Acids Res 2008; 36(suppl_2):W509–W512. doi:10.1093/nar/gkn202.
43. Nielsen M, Lundegaard C, Lund O, et al. The role of the proteasome in generating cytotoxic T-cell epitopes: insights obtained from improved predictions of proteasomal cleavage. Immunogenetics 2005;57(1-2): 33–41. doi:10.1007/s00251-005-0781-7.
44. Bhasin M, Raghava G. Analysis and prediction of affinity of TAP binding peptides using cascade SVM. Protein Sci 2004;13(3):596–607. doi:10.1110/ps. 03373104.
45. Bhasin M, Raghava G. Pcleavage: an SVM based method for prediction of constitutive proteasome and immunoproteasome cleavage sites in antigenic sequences. Nucleic Acids Res 2005;33(suppl_2): W202–W207. doi:10.1093/nar/gki587. 14 A. A. BAHRAMI ET AL.
46. Singh H, Raghava G. ProPred: prediction of HLADR binding sites. Bioinformatics 2001;17(12): 1236–1237. doi:10.1093/bioinformatics/17.12.1236.
47. Ponomarenko J, Bui H-H, Li W, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinform 2008;9(1):514. doi:10.1186/ 1471-2105-9-514.
48. Feldhahn M, Thiel P, Schuler MM, et al. EpiToolKit—a web server for computational immunomics. Nucleic Acids Res 2008;36(suppl_2): W519–W522. doi:10.1093/nar/gkn229.
49. Lundegaard C, Lund O, Buus S, et al. Major histocompatibility complex class I binding predictions asa tool in epitope discovery. Immunology 2010;130(3): 309–318. doi:10.1111/j.1365-2567.2010.03300.x.
50. Berman H, Henrick K, Nakamura H, et al. The worldwide Protein Data Bank (wwPDB): ensuring a single, uniform archive of PDB data. Nucleic Acids Res 2007;35 (suppl_1): D301–D303. doi:10.1093/nar/ gkl971.