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Multi-perspectives and challenges in identifying B-cell epitopes

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Abstract

The identification of B-cell epitopes (BCEs) in antigens is a crucial step in developing recombinant vaccines or immunotherapies for various diseases. Over the past four decades, numerous in silico methods have been developed for predicting BCEs. However, existing reviews have only covered specific aspects, such as the progress in predicting conformational or linear BCEs. Therefore, in this paper, we have undertaken a systematic approach to provide a comprehensive review covering all aspects associated with the identification of BCEs. First, we have covered the experimental techniques developed over the years for identifying linear and conformational epitopes, including the limitations and challenges associated with these techniques. Second, we have briefly described the historical perspectives and resources that maintain experimentally validated information on BCEs. Third, we have extensively reviewed the computational methods developed for predicting conformational BCEs from the structure of the antigen, as well as the methods for predicting conformational epitopes from the sequence. Fourth, we have systematically reviewed the in silico methods developed in the last four decades for predicting linear or continuous BCEs. Finally, we have discussed the overall challenge of identifying continuous or conformational BCEs. In this review, we only listed major computational resources; a complete list with the URL is available from the BCinfo website (https://webs.iiitd.edu.in/raghava/bcinfo/).

KEYWORDS

B-cell epitope prediction, class-specific epitopes, conformational B-cell epitope, databases, deep learning, linear B-cell epitope, machine learning

1 | INTRODUCTION

Organisms have evolved complex defense systems that rely on a series of protective mechanisms to recognize and eliminate foreign substances. The immune system consists of two components, namely the non-specific innate immunity and the antigen-specific adaptive immunity, which work synergistically but differ in their mechanisms of action. Innate immunity acts as the initial defense mechanism and relies on pattern recognition receptors expressed on various immune and non-immune cells to detect pathogen-associated molecular patterns. This recognition triggers a response that activates the adaptive immune system. In turn, adaptive immunity generates responses that are based on the specific recognition of antigens by receptors expressed on the surfaces of B-lymphocytes (resulting in the humoral immune response) and T-lymphocytes (resulting in the cell-mediated immune response) (Chaplin, 2010; Tomar & De, 2014).

B-cells are a key component of the adaptive immune system and play a critical role in providing long-term protection against pathogens, achieved by producing plasma cells and memory cells. Plasma cells have specific receptors called antibodies, an essential component of the antigen–antibody (Ag–Ab) interaction in the humoral immune response. Although antigens are typically larger, antibodies or B-cell receptors specifically recognize and bind to certain regions of antigens called antigenic determinants or epitopes (Jespersen et al., 2019). Antibodies recognize these regions through interactions with their binding site, or paratope, and play a vital role in eliciting immune responses (Jespersen et al., 2019). Therefore, accurately characterizing and identifying B-cell epitopes (BCEs) is crucial for developing epitope-based vaccines (Russi et al., 2018), disease prevention, and immunological diagnostic tools (Schellekens et al., 2000). It is worth noting that therapeutic antibodies, which have demonstrated high efficacy, selectivity, and safety, have been extensively studied and reported in the literature (Kam et al., 2012; Manavalan et al., 2018; Potocnakova et al., 2016; Schneider et al., 2014; Siman-Tov et al., 2013).

BCEs are clusters of amino acids that are surface accessible and belong to two primary classes based on their spatial structure: continuous (linear or sequential) and discontinuous (nonlinear or conformational) (Atassi & Smith, 1978; Jespersen et al., 2019; Potocnakova et al., 2016). The sequence determines antibody binding to sequential epitopes, and it does not depend on the tertiary structure of the antigen. Therefore, sequential epitopes are small segments of a protein called antigenic regions. In contrast, antibody binding to conformational epitopes relies on the antigen's three-dimensional (3D) structure (Benjamin et al., 1984; Gershoni et al., 2007; Kulkarni-Kale et al., 2005). Approximately 90% of total BCEs are discontinuous, which means that the residues in the sequence are far from each other and come close together in the vicinity by protein folding, forming a functional antigenic determinant region (Kringelum et al., 2013). Hence, without having accurate high-resolution structural information on the Ag–Ab complex, identifying discontinuous epitopes is challenging (Haste Andersen et al., 2006; Najar et al., 2017). Studies have also suggested that several groups of continuous epitopes lie adjacent to discontinuous epitopes, which blurs the line between continuous and discontinuous epitopes (Galanis et al., 2021; Van Regenmortel, 2006). The accurate determination of conformational BCEs is highly dependent on the 3D structure of the antigen (Jespersen et al., 2019; Raoufi et al., 2020; Sharon et al., 2014). In the era of next-generation sequencing, where numerous pathogens have been sequenced due to advancements in sequencing technology. There is a need to identify BCEs (antibody interacting residues) in an antigen at the genome scale for various immunological applications (Galanis et al., 2021). Experimental techniques such as nuclear magnetic resonance (NMR), western blotting, X-ray crystallography, and many more remain the gold standard for identifying epitopes but are time-consuming and costly (Abbott et al., 2014; Arnold et al., 2018; Ashford et al., 2021; Frank, 2002; Jespersen et al., 2019; Potocnakova et al., 2016). In contrast, computational approaches are easy to develop and efficient in terms of cost and time. They can be easily implemented at the genome scale, which is not possible with experimental techniques. To rapidly identify potential epitopes, new computational methods must be developed to enhance precision by selecting specific regions for effective epitopes with high probability (Ansari & Raghava, 2010; Kozlova et al., 2018; Manavalan et al., 2018; J. Zhang et al., 2014).

There have been limited studies reviewing prediction tools that cover all categories of BCEs. In 2013, Ansari and Raghava presented all the available in silico tools for BCE prediction. Subsequently, Potocnakova et al. (2016) reviewed the experimental mapping methods for BCEs as well as the existing databases, resources, and online available in silico tools. A review performed by Sanchez-Trincado et al. (2017) highlighted the antigenic recognition of both B and T cells and listed the most relevant prediction tools for the same. Additionally, there are reviews that focus solely on prediction tools for either linear BCEs, such as the study by Wang and Pai (2014) or conformational BCEs, as explored by P. Sun et al. (2013).

Over the past few decades, numerous computational methods for predicting conformational or linear BCEs have been published in the scientific literature. The combinational approach of bioinformatics and epitope mapping technologies has contributed significantly to immunotherapy and subunit vaccines (De Groot et al., 2001; Lo et al., 2021; Rueckert & Guzmán, 2012). Therefore, this review offers researchers a comprehensive understanding of the current techniques utilized for mapping BCEs. It will also provide an overview of the latest and up-to-date epitope databases, highlight recently developed prediction methods, and explore the publicly available tools in this research field (Potocnakova et al., 2016).

2 | THE EXPERIMENTAL APPROACH FOR BCE IDENTIFICATION

Identifying regions on the antigen surface that are specifically recognized by the complementarity of antibodies is known as epitope mapping, and this can be done by using numerous experimental methods. In this review,

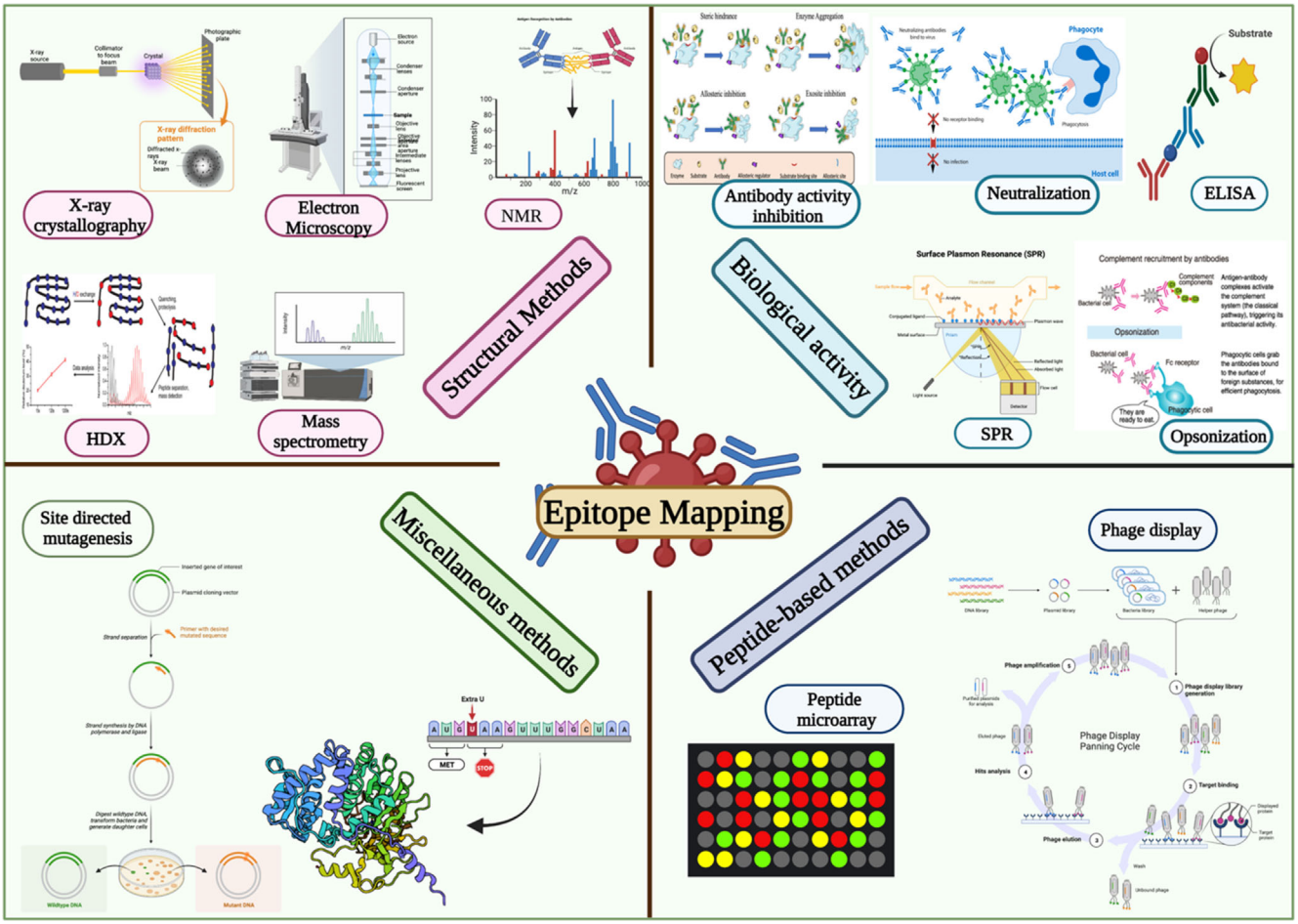

FIGURE 1 Pictorial representation of experimental methods for mapping BCEs. BCE, B-cell epitope.

we divide these experimental techniques into the following categories: (i) structural methods, (ii) biological activities, (iii) peptide-based methods, and (iv) miscellaneous methods (see Figure 1). The approximation count of B-cell discovered using these experimental methods is graphically represented in Figure 2 (Vita et al., 2019).

2.1 | Structure-based methods

In this approach, the structure of the antibody–antigen (Ab–Ag) complex is determined using an experimental technique. Various sophisticated software is employed to identify the antibody interacting residues on the antigen, and these residues or regions are assigned as conformational BCEs. Over the years, several techniques have been developed for determining the tertiary structure of proteins, especially the structure of Ab–Ag complexes. The following are major techniques: X-ray crystallography, electron microscopy (EM), NMR, mass spectrophotometer (MS), and Hydrogen-deuterium exchange (HDX). X-ray crystallography was one of the initial techniques used for

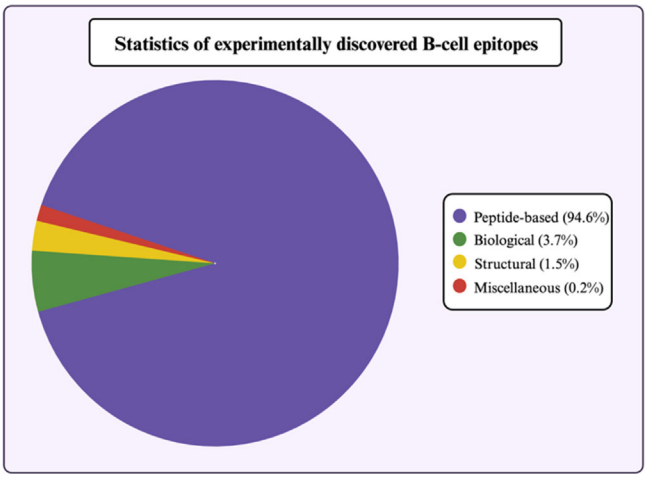

FIGURE 2 A graphical description of B-cell epitopes unveiled through experimentation.

epitope mapping. It involves the use of highly purified co-crystals of an Ag–Ab complex. The structure of the epitope is inferred by interpreting the 3D coordinates of the complex and identifying specific amino acids

involved in the interaction. While this is the most effective technique for determining the tertiary structure of proteins, it is not always feasible to solve all complex structures (Caliandro et al., 2013).

EM is another option for determining the structure of Ab–Ag complexes. Initially, using EM for epitope mapping was challenging due to its low resolution. However, this limitation was overcome in 2013 with the introduction of alternate cryo-EM. This technique is less complex and does not require purified Ag–Ab complex components or crystals. Several studies have utilized this technique to map BCEs in HIV, HPV, and poliovirus for vaccine production (Bannwarth et al., 2015; J. H. Lee, Leaman, et al., 2015; H. Lee, Brendle, et al., 2015). NMR is a technique used to provide the 3D structure of the Ag–Ab complex by measuring the distance and angle between amino acids. It involves subjecting the complex to a magnetic field and electromagnetic radiations, which provide information about the dynamics of the complex in solution. Unlike X-ray crystallography, NMR does not require crystals. However, NMR has significant limitations, such as its applicability being restricted to small continuous proteins and peptides (below 30 kDa), and the purity and concentration of the complex should be high (Ahmad et al., 2016). Additionally, because of the requirement of a high level of expertization and technical hurdles, this method is not commonly used (Abbott et al., 2014).

Another crucial technique in epitope mapping within this category is Mass spectrometry (MS). MS has introduced new strategies to elucidate the higher-order structure of antibodies, enabling the identification of specific residues that constitute the binding epitope and paratope, particularly in the case of discontinuous epitopes (Opuni et al., 2018). It can be employed using the limited proteolysis method, where the antigen is fragmented using different proteases. MS detects the generated fragments to reveal the fragments bound to the antibody. Another approach that can be employed is the epitope excision method, where antigens are exposed to beads coated with antibodies, followed by proteolytic digestion and washing to remove non-epitope fragments. Finally, the bound fragments are identified using LC–MS or MALDI/MS techniques (Suckau et al., 1990; Temporini et al., 2014). One major limitation of MS in epitope mapping is its resolution, as it can identify longer peptide sequences, typically ranging from 30 to 60 residues (Baerga-Ortiz et al., 2002).

Similarly, HDX is an emerging field in epitope mapping that focuses on identifying the interaction sites between antigens and antibodies. In this method, the amide hydrogen atoms within the protein backbone are substituted with deuterium ions present in a deuterium oxide solution. This substitution allows deuterium to serve as a tool for studying protein structure, dynamics, and interactions over a specific period of time. By analyzing variations in deuterium incorporation, valuable information can be obtained regarding the protein's characteristics. HDX, when combined with mass spectrometry (HD-MS), overcomes the shortcomings of standard MS methods and offers several advantages for epitope mapping. These advantages include low sample consumption, high throughput, rapid analysis, high sensitivity, and no theoretical upper molecular weight limit. Despite its benefits, HDX technology has several limitations. It cannot identify changes in single amino acid residue exchanges, and it may lead to allosteric effects caused by antibody binding, resulting in conformational changes in the antigen region. These changes can introduce differences in HDX, which can potentially bias the epitope recognition process when relying on HDX-MS (H. Sun et al., 2021).

2.2 | Biological activity

Epitope mapping techniques that rely on the biological activity of the Ag–Ab interaction include several methods, such as inhibition of antibody activity, neutralization, agglutination, opsonization reactions, and binding ability of the antibodies toward specific antigens or their fragments. These biological reactions play a crucial role in identifying epitopes. Inhibition of antibody activity involves studying the effect of specific enzymes on antibody function, which can help pinpoint the regions or epitopes targeted by the antibodies. Binding methods used in functional epitope mapping include immunological assays where antigenic peptides are immobilized on a solid support, and the binding of antibodies is detected by ELISA, Western blot, and ELISPOT (Potocnakova et al., 2016). These approaches offer the advantage of not requiring expensive equipment and can quantify the immune response toward a specific epitope. Surface plasmon resonance technology is a sensitive method for detecting protein interaction binding kinetics, affinity, and specificity. However, it does not provide detailed information about the epitopes involved in the interaction (Fivash et al., 1998; Patching, 2014).

2.3 | Peptide-based methods

In this method to map the BCEs, an approach of overlapping peptides is employed to identify the B-cell putative epitopes or mimotopes (peptides mimicking an epitope on the corresponding antigen). This is done by screening

random peptide libraries for antibody binding (Moreau et al., 2006; Ponomarenko & Marc, 2008). The peptide libraries are either chemically synthesized or generated by display methods corresponding to overlapping or randomized regions of a certain protein. Techniques including PEPSCAN, SPOTS, or peptide microarray are used to produce synthetic peptides. Peptide microarrays offer an advantage over others by enabling the screening of a large number of peptides with minimal reagent requirement (Santona et al., 2002; Zander et al., 2007).

An alternate approach involves using phages to generate biological peptide libraries to produce and display the peptides coupled with peptide-encoding nucleic acid sequence. Affinity selection through biopanning allows testing the binding capacity of displayed peptides to the monoclonal antibody of interest, providing insights into the amino acids crucial for antibody binding (Potocnakova et al., 2016). Biologically displayed peptides possess a high number (up to 10; Potocnakova et al., 2016) and length variation of displayed peptides. An advantage common to all peptide methods is that they do not require the antigen itself, which can be important for purifying "unique" antigens (Morris, 2007). They require less specialized equipment and expertise and also are well-suited for situations where the epitope is a linear peptide sequence but may not identify complex conformational epitopes involving high-order structures (Hensen et al., 2014; Nilvebrant & Rockberg, 2018).

2.4 | Miscellaneous methods

Antigen modification through mutagenesis is a rapid method for epitope mapping. It involves replacing individual amino acid residues that constitute a functional epitope, leading to a loss of antibody binding. Mutations can be introduced randomly or through site-directed mutagenesis. In site-directed mutagenesis, specific chemical reagents are used to introduce mutations into targeted antigen sequences. The binding ability of the mutated antigen to the corresponding antibody is then tested. Side chains play a crucial role in Ag–Ab complexes, and alanine scanning mutagenesis is a technique that sequentially substitutes each non-alanine residue with alanine to determine the contribution of each residue's side chain to antibody binding (Lo Conte et al., 1999). When combined with the phage display technique, site-directed mutagenesis has shown successful results in epitope mapping. This combination enables the identification and characterization of epitopes with high specificity (Gershoni et al., 2007; Wind et al., 2001).

Overall, biological activity-based methods offer valuable insights into the impact of specific residues of amino acid on the binding strength of the Ag–Ab complex. They are faster and more cost-effective than structural methods (Potocnakova et al., 2016). However, these methods have limitations regarding identifying all important epitopes. Figure 3 in the referenced article depicts the limitations of some epitope mapping methods (Coales et al., 2009; Malito et al., 2013; Mayer & Meyer, 2001; Obungu et al., 2009; Pandit et al., 2012; Rux & Burnett, 2000; P. Sun et al., 2011; Q. Zhang et al., 2011).

3 | BCEs: A HISTORICAL FRAMEWORK AND DATABASE OVERVIEW

3.1 | History of BCEs identification

The identification of epitopes has a long and rich history in research and discovery, spanning several decades. It is because epitopes are crucial in developing synthetic peptides that can elicit antibodies capable of neutralizing the infectivity of pathogens containing the antigenic determinant region (Ponomarenko & Van Regenmortel, 2009; P. Sun et al., 2013; Van Regenmortel, 2006). The work of Hoops and Woods in the 1980s and 1990s, which involved predicting linear epitopes using propensity scales to calculate hydrophilicity residues with simple algorithms, was pioneering and garnered significant attention from the scientific community in the field of BCE prediction. Subsequently, several techniques have been proposed for predicting linear epitopes, employing direct prediction methods that rely on the physicochemical characteristics of individual amino acid residues commonly observed in known epitopes. These characteristics include attributes such as β-turn propensity, charge, flexibility, antigenicity, amino acid frequency, surface accessibility, and secondary structure (El-Manzalawy et al., 2008; Garnier et al., 1978; Haste Andersen et al., 2006; Hopp & Woods, 1981; Karplus & Schulz, 1985; Parker et al., 1986; Pellequer et al., 1993; Welling et al., 1985; Yang & Yu, 2009).

Traditionally, propensity scales have been used for predicting BCEs. These methods consider the physicochemical properties of residues which can be easily computed even by a calculator and are easy to implement and understand, which makes them a popular method. Several studies have demonstrated that these methods have limited precision power (Blythe & Flower, 2005; Giacò et al., 2012; Kulkarni-Kale et al., 2005; Pellequer et al., 1991; Pellequer & Westhof, 1993; Söllner & Mayer, 2006). To overcome the limitations of traditional methods, machine learning (ML)-based techniques have been proposed. These ML models are trained using

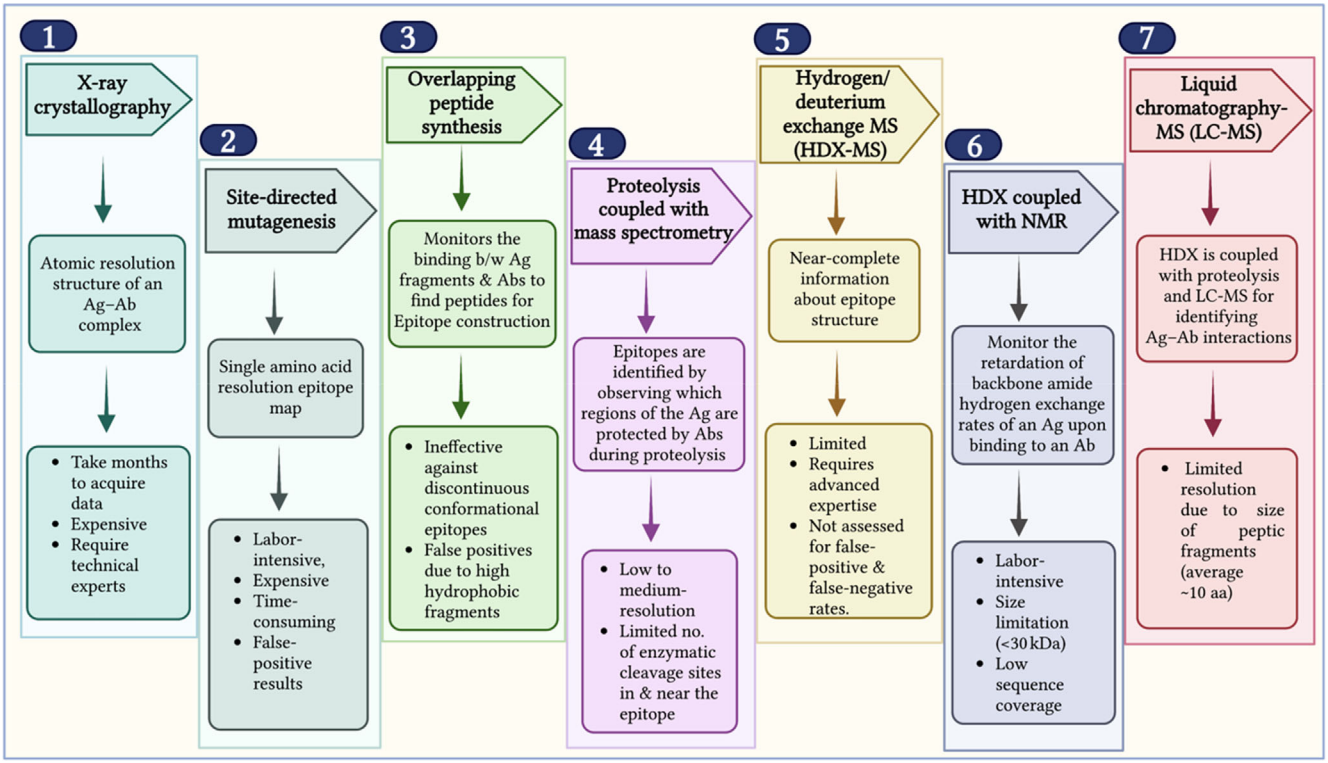

FIGURE 3 Limitations of some commonly used B-cell epitope mapping methods.

feature vectors extracted from various sources, including propensity scales, protein sequences, and 3D structures (Ponomarenko & Bourne, 2007). Figure 4 illustrates some general methods publicly available for BCE prediction.

In general, most of the epitopes are conformational epitopes; only around 10% of epitopes are continuous or linear, even though linear epitopes are one tenth of the total epitopes. Several methods have been developed to predict the discontinuous epitopes, but they are few in number compared to linear BCE prediction methods. Predicting linear BCEs from the antigenic sequence is relatively straightforward since an epitope represents a specific segment or region within a protein. In addition, designing subunit vaccines based on linear epitopes is practically accessible (Sanchez-Trincado et al., 2017).

In contrast, only a few tools exist for predicting conformational BCEs, which rely on information from protein structures and the spatial arrangement of amino acids to make predictions. In 1984, Westhof et al. made initial experimental attempts for epitope prediction based on protein 3D structures (Westhof et al., 1984). Subsequently, Jones and Thornton developed a method for predicting discontinuous epitopes using structural properties in 1997 (Amit et al., 1986; Jones & Thornton, 1997). Several protein–protein docking algorithms have been utilized for epitope prediction based on predefined complimentary 3D structures of epitope and paratope (Halperin et al., 2002). In 2005, Kulkarni-Kale developed the first conformational epitope prediction tool, CEP, which predicted sequential BCEs using structural information (Kolaskar & Kulkarni-Kale, 1999; Kulkarni-Kale et al., 2005). However, despite significant advancements in defining protein structures, numerous tools have been developed for predicting continuous or discontinuous BCEs, while the overall performance of these tools is far from precision. This could be due to difficulties in extracting valuable features from antigen 3D structures or inadequate non-redundant data sets. Thus, the scientific community requires more extensive algorithms and data sets that can define features and computational tools for predicting BCEs with high precision.

3.2 | Repositories BCEs

In this review, we present a concise summary of well-known databases dedicated to BCEs, serving as valuable resources for researchers seeking access to data to develop computational tools. The availability of experimental data plays a crucial role in enhancing the accuracy of BCE prediction. With advancements in biological techniques, a vast amount of BCE-related information is continuously being released and made accessible through

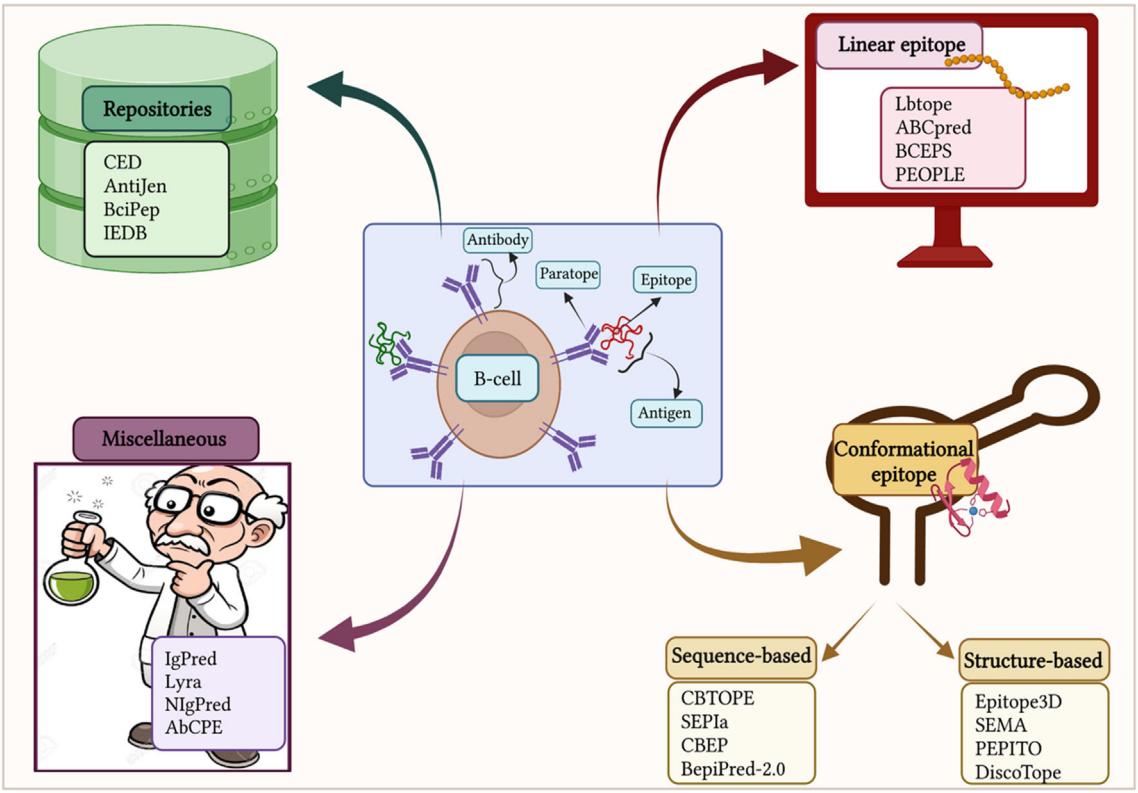

FIGURE 4 Depiction of some available B-cell prediction methods.

online platforms or scientific publications. Figure 5 displays some of the prominent databases for BCEs.

Bcipep emerged as one of the early databases designed specifically for managing continuous BCEs (Saha et al., 2005). It is a comprehensive database that encompasses a broad spectrum of pathogens, containing 1797 immunogenic, 763 immunodominant, and 471 null-immunogenic epitopes. In order to facilitate users, Bcipep provides several web-based applications that include keyword search, epitope mapping, and BLAST search. In contrast, the HIV molecular immunology database focuses exclusively on the HIV virus, containing nearly 11,361 HIV associate B-/T-cell epitopes (Liu & Zhang, 2014). Another old database is AntiJen 2.0, developed for maintaining a wide range of information required for immunotherapy or subunit vaccines. It provides comprehensive information on MHC binders, TCR–MHC complexes, and BCE molecules (Toseland et al., 2005). IEDB is One of the most commonly used and authoritative databases in the field of immunology, established in 2004 (Peters et al., 2005; Vita et al., 2019). Protein Data Bank (PDB) is one of the essential resources in structural biology that maintain the structure of proteins and their complexes (Berman et al., 2000). The complex structure of antibodies and antigens is used to identify antigenic residues/regions in an antigen and discontinuous BCEs. Another important database for discontinuous epitopes based on the structure is CED, a conformational epitope database containing 293 manually curated entries (Huang & Honda, 2006). Epitome is a database of all known antigenic epitopes inferring from the Ag–Ab complexes and the antibodies that interact with them (Schlessinger et al., 2006). Some other available databases used by the researchers in this field include SEDB, SDAP, and FLAVIdB (Ivanciuc et al., 2003; Olsen et al., 2011; Sharma et al., 2012).

4 | CONFORMATIONAL BCE PREDICTORS

Approximately 90% of the BCEs are discontinuous in nature which makes it challenging to imitate them for the development of antibody therapeutics, epitope-based vaccines, and immunological tools (Ferdous et al., 2019). Conformational BCE tools can be classified into two types: conformational sequence-based prediction tools and conformational structure-based tools.

4.1 | Structure-based predictors

To advance the performance of conformational BCEs prediction in an antigen from its structure, several studies

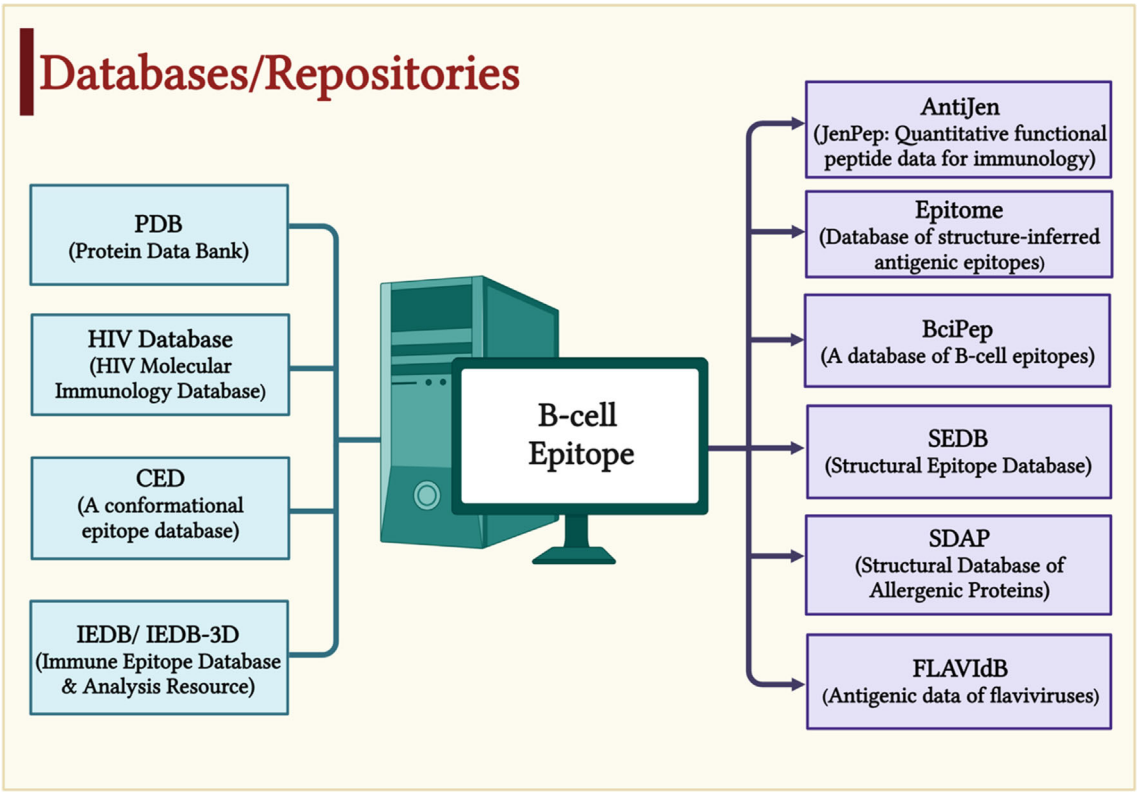

FIGURE 5 Available databases of B-cell epitope prediction.

have proposed methods in the past, including CEP, Discotope, Pepito, and Epitopia (Haste Andersen et al., 2006; Kulkarni-Kale et al., 2005; Rubinstein et al., 2009; Sweredoski & Baldi, 2008). These techniques utilized the 3D structural information of an antigen, as well as the properties of amino acids, surface accessibility, residue clustering, and spatial information. A better understanding of the 3D structure of antigen help to identify antigenic residues in proteins with high accuracy (Ferdous et al., 2019). J. Sun et al. (2009) used the concept of "unit patch of residue triangle" and proposed a method SEPPA to characterize the local spatial context in protein surfaces. SEPPA 2.0 (Qi et al., 2014) consolidates the AAindex (amino acid index) characteristics via artificial neural networks (ANNs) algorithm. However, SEPPA 3.0 additionally included glycosylation triangles and a glycosylation-related AAindex, which was similarly consolidated using ANN (Solihah et al., 2020). Table 1 provides a summary of further structure-based prediction methods.

4.2 | Sequence-based predictors

The prediction of linear BCEs relies solely on the primary sequence of an antigen, without requiring the availability of 3D structures. While structure-based predictors may predict conformational BCEs with high accuracy, their limitation was the requirement of the tertiary structure of the antigen. Identification of the tertiary structure of an antigen is expensive, tedious, and time-consuming. Ansari and Raghava proposed a method, CBTOPE (Ansari & Raghava, 2010), to overcome these limitations, which predicts constitutive conformational BCEs from the antigen's primary sequence (Ansari & Raghava, 2010; P. Sun et al., 2013). In this method, they introduced the concept of composition profile of patterns (CPP) and constructed the model using binary profile (BPP) and physiochemical profile of the patterns (PPP). Subsequently, several other methods have been developed including CBEP (J. Zhang et al., 2014), BepiPred-2.0 (Jespersen et al., 2017), and SEPIa (Dalkas & Rooman, 2017). A list of methods developed by different groups using a wide range of features is shown in Table 2.

5 | LINEAR BCEs PREDICTION METHODS

Early methods of predicting BCEs involved the use of propensity scales, which were determined experimentally (Hopp & Woods, 1981). In 1984, researchers established a

TABLE 1 List of methods developed for the prediction of discontinuous or conformational epitopes in an antigen.

Name	Year	Description	Performance measure					Data set
			Acc	AUC	MCC	Spec	Sens	
CEP (Kulkarni-Kale et al., 2005)	2005	Identify both sequential and conformational B-cell epitopes in an antigen from its 3-D structure.	75%	-	-	-	-	CEP data set
DiscoTope (Haste Andersen et al., 2006)	2006	Specifically developed for predicting discontinuous B-cell epitopes with specificity.	-	-	-	95%	15.5%	SACS database
ElliPro (Ponomarenko et al., 2008)	2008	Prediction and visualization of antibody epitopes in a given protein sequence or structure.	0.84	0.732	-	-	0.601	Ponomarenko data set
PEPITO (Sweredoski & Baldi, 2008)	2008	A method based on amino-acid propensity scores and half-sphere exposure values at multiple distances.	-	75.4	-	-	-	Discotope, Epitome
SEPPA (J. Sun et al., 2009)	2009	A unit patch of residue triangle at protein surface is used for predicting spatial epitope in a protein.	-	0.742	-	0.707	0.58	SEPPA's data set, Epitome, Discotope
EPCES (Liang et al., 2020)	2020	Prediction of B-cell epitopes at protein surface using different types of conformation information.	-	0.632.	-	69.50%	47.80%	EPCES Data set 1, EPCES Data set 2, EPCES Data set 3
EPSVR (Liang et al., 2010)	2010	An SVM-based model developed using six scoring terms for predicting antigenic epitopes.	-	0.597	-	-	-	Ponomarenko data set, EPCES data set, benchmark2,[a]CED
EPMeta (Liang et al., 2010)	2010	A meta server that utilizes five prediction servers for predicting B-cell epitopes.	-	0.638	-	-	-	EPSVR data set
DiscoTope 2.0 (Kringelum et al., 2012)	2012	An improved version of DiscoTope, for predicting discontinuous epitopes with high precision.	-	0.712–0.727	-	-	-	DiscoTope data set, DiscoTope2.0 data set
SEPPA 2.0 (Qi et al., 2014)	2014	It is an improved version of SEPPA and integrates relative ASA of unit patch and amino acid index.	-	0.823,[b] 0.745[c]	-	0.865,[b] 0.754[c]	0.799,[b] 0.734[b]	SEPPA2.0, predictor, EPMeta, DiscoTope2.0
Seppa 3.0 (Zhou et al., 2019)	2019	It is an improved version of SEPPA 2.0 and allows predicting epitope in glycoprotein antigens.	0.665	0.749	-	-	-	SEPPA3.0's data set
CluSmote (Solihah et al., 2020)	2020	It is a combination of a cluster-based under sampling and synthetic minority oversampling technique.	-	0.766	-	-	-	Rubinstein's data set, DiscoTope2.0 data set, SEPPA 3.0 data set
SEMA (Shashkova et al., 2022)	2022	A deep learning-based method for predicting conformational epitopes using sequence and structure.	-	0.76	-	-	-	SEMA data set
Epitope3D (da Silva et al., 2021)	2021	A machine learning method that uses concept of graph-based signatures to model epitope regions	-	-	0.55	-	-	-

Abbreviation: CED, Conformational Epitope Database.

[a]Protein-Protein Docking Benchmark 2.0.

[b]Subcellular localization/Species: Secretory/mus.

[c]Subcellular localization/Species: Unspecified/unspecified.

TABLE 2 List of available conformational sequence-based predictors.

Name	Year	Description	Performance measure					Data set
			Acc	AUC	MCC	Sens	Spec	
Epitopia (Rubinstein et al., 2009)	2009	A machine learning-based method for predicting antigenic regions in an antigen sequence or structure.		0.59				ElliPro data set
CBTOPE (Ansari & Raghava, 2010)	2010	An SVM-based method for predicting conformational B-cell epitopes and antigenic residues in a protein sequence.	86.59%	-	0.73	83.13	90.06	CBTOPE data set, Ponomarenko data set
CBEP (Zhang et al., 2014)	2014	An ensemble method that utilizes spatial clustering for predicting discontinuous epitopes in protein from its sequence	-	0.721,[a] 0.703[b]	-	-	-	Rubinstein's data set,[a] Liang's data set[b]
BepiPred-2.0 (Jespersen et al., 2017)	2017	A random forest-based model trained on antibody–antigen complex structure for predicting epitope from sequence.	-	0.62	-	-	-	BepiPred2.0 data set
SEPIa (Dalkas & Rooman, 2017)	2017	Combination of naïve Bayesian and random forest classifier for predicting conformational epitopes from protein sequence.	-	0.65	-	-	-	SEPIa data set

[a]Data type: Bound dataset.
[b]Data type: Unbound dataset.

correlation between crystallographic temperature factors and several known continuous epitopes of proteins such as tobacco mosaic virus protein, myoglobin, and lysozyme. They also found a relationship between antigenicity, solvent accessibility, and flexibility of antigenic regions in proteins. Thornton and colleagues proposed a technique for identifying continuous epitopes in the protein regions that protrude from the proteins' globular surface. They discovered that regions with high protrusion index values corresponded to the experimentally determined continuous epitopes in myoglobin, lysozyme, and myohaemerythrin. This information can be beneficial in predicting BCEs (Ponomarenko et al., 2008).

5.1 | The era of propensity scales-based methods

The classical approach of predicting linear BCEs is based on a propensity scale of physicochemical properties. In 1981, Hoops and Wood proposed the first propensity scales-based linear BCE prediction method, which utilized the Levitt hydrophilicity scale. Afterward, several other propensity scales methods were introduced, such as those using exposed surface area (Janin & Wodak, 1978), secondary structure (Levitt et al., 1978), polarity (Ponnuswamy et al., 1980), flexibility (Karplus et al., 1985), accessibility (Emini et al., 1985), hydrophilicity (Parker et al., 1986), antigenic scale (Kolaskar et al., 1990), and turns (Pellequer et al., 1991). To further improve the accuracy of epitope prediction, methods tried combinations of different properties (e.g., hydrophilicity, flexibility, and polarity); the following are major methods under this category PEOPLE, PREDITOP, BEPITOPE, and BcePred (Table 3). In 2006, a method was developed by integrating the propensity scale with the hidden Markov model (Larsen et al., 2006; El-Manzalawy et al., 2010).

5.2 | ML-based methods

It has been shown in number of studies that propensity scale-based methods have their own limitations (Blythe & Flower, 2005). Even methods developed based on propensity scales of all physicochemical properties have poor performance. To overcome this limitation,

TABLE 3 List of available methods of B-cell epitope prediction based on physicochemical properties.

Name	Year	Description	Performance measure					Data set
			Acc	AUC	MCC	Sens	Spec	
Janin and Wodak (Janin et al., 1978)	1978	Prediction of B-cell epitopes, a protein based on conformation of amino acid side-chains exposed surface scale	-	-	-	-	-	Janin data set
Levitt Method (Levitt et al., 1978)	1978	Conformational preferences of amino acids derived from protein structure used for prediction.	-	-	-	-	-	Levitt data set
Ponnuswamy (Ponnuswamy et al., 1980)	1980	Hydrophobic packing and spatial arrangement of amino acid residues in globular proteins Polarity	-	-	-	-	-	Ponnuswamy data set
Hopp and Woods (Hopp & Woods, 1981)	1981	A hydrophilicity-based scale for predicting antigenic determinants in a protein.	-	-	-	-	-	-
Karplus and Schulz (Karplus & Schulz, 1985)	1985	A flexibility-based scale for predicting B-cell epitopes.	-	-	-	-	-	Karplus data set
Emini Method (Emini et al., 1985)	1985	Accessibility-based score for identification.	-	-	-	-	-	-
Parker Method (Parker et al., 1986)	1986	Antigenic region prediction based on hydrophilic, accessible, or mobile regions.	-	-	-	-	-	Brookhaven Parker data set
Kolaskar Method (Kolaskar & Tongaonka, 1990)	1990	Prediction of antigenic determinants in a protein based on physicochemical properties of amino acid residues.	75%	-	-	-	-	Kolaskar data set
Pellequer Method (Pellequer et al., 1991)	1991	Predicting of continuous epitopes in a protein from its primary structure.	-	-	-	-	-	Pellequer data set
PREDITOP (Pellequer & Westhof, 1993)	1993	A program for predicting antigenicity, which uses 22 normalized scores based on physicochemical properties.	60%	-	-	-	-	-
PEOPLE (Alix AJ, 1999)	1999	It analyzed all residual properties used in previous methods to predict linear B-cell epitopes.	-	-	-	-	-	PEOPLE data set
BEPITOPE (Odorico & Pellequer, 2003)	2003	It is an updated version of PREDITOP which uses many properties including turns in a protein.	-	-	-	-	-	-
BcePred (Saha & Raghava, 2004)	2004	Prediction of continuous B-cell epitopes using combination of all physicochemical properties.	58.70%	-	-	56%	61%	Bcipep database
BepiPred (Larsen et al., 2006)	2006	This method combines hidden Markov model with propensity scale methods for predicting linear B-cell epitopes.	-	0.671	-	-	-	AntiJen data set, HIV data set, Pellequer data set
AAPPred (Davydov & Tonevitskii, 2009)	2009	Prediction of linear B-cell epitopes using amino acid pair score of residue properties.	-	-	-	-	-	Bcipep, FIMM, AntiJen, AAPPred data set

TABLE 4 Machine learning-based linear B-cell epitopes prediction methods.

Name	Year	Description	Performance measure					Data set
			Acc	AUC	MCC	Spec	Sens	
ABCpred (Saha & Raghava, 2006)	2006	First time artificial neural network has been used for predicting continuous B-cell epitopes.	65.93%	-	0.3187	64.71	67.14	Bcipep database
Söllner (Sollner & Mayer, 2006)	2006	Machine learning-based model using propensity scales for predicting epitopes.	-	-	-	0.58	0.88	Bcipep database, FIMM database
FBCPred (El-Manzalawy, 2008)	2008	Prediction of B-cell epitope of variable length using SVM and compositional features.	73.37	0.812	-	72.67	74.08	Bcipep database
BCPREDS (El-Manzalawy, 2008)	2008	SVM-based method using customized kernel string for predicting B-cell epitopes.	67.9	0.758	-	63.2	72.61	Bcipep database, ABCPred data set
COBEpro (Sweredoski & Baldi, 2008)	2009	Two-stage prediction based on propensity of antigenic region and residues.	78.00%	0.829	-	95.1	60.9	Bcipep database, Pellequer, HIV, Chen data set, BCPRED data set
BayesB (Wee et al., 2010)	2010	SVM-based prediction of linear B-cell epitopes using Bayes-based feature extraction.	74.50%	0.84	-	68%	81%	BCPRED data set, Chen data set
LEPS (Wang et al., 2011)	2011	Physicochemical propensities-based SVM model for predicting linear epitopes.	72.52%	-	10.36%	84.22%	-	Bcipep and Chen data set, AntiJen data set, HIV data set, PC data set, AHP data set
BEOracle[#]/ BROracle (Wang et al., 2011)[##]	2011	An SVM-based method developed using tripeptide similarity and propensity score.	96.88%[#] 75.26%[##]	0.83[#] 0.81[##]	-	-	-	Bcipep database AntiJen data set, Protein Atlas database, BEOracle data set
SVMTriP (Yao et al., 2012)	2012	An SVM-based method developed using tripeptide similarity and propensity score.	-	0.702	-	-	80.10%	SVMTriP data set
BEST (Gao et al., 2012)	2012	A sliding window method developed using SVM.	74.5%	0.81	0.53	0.929	0.561	Chen data set, BciPep database, BCPRED data set, BEST data set, Epitopia (SEQ194) data set
LBTope (Singh et al., 2013	2013	A method trained on experimentally validated B-cell epitopes and non-B-cell epitopes.	81.24,[a] 64.86,[b] 78.82,[c] 66.7,[d] 82.33[e]	0.88,[a] 0.69,[b] 0.84,[c] 0.73,[d] 0.91[e]	0.61,[a] 0.3,[b] 0.57,[c] 0.33,[d] 0.64[e]	81.67,[a] 63.97,[b] 77.5,[c] 67.24,[d] 81.01[e]	80.5,[a] 65.75,[b] 80.9,[c] 66.0,[d] 84.62[e]	LBTope data set
EPMLR (Lian et al., 2014)	2014	A sequence-based method for predicting epitopes.	-	0.728			81.80%	BEOracle data set, SVMTriP data set, LFNR[b] data set
LBEEP (Saravanan & Gautham, 2015)	2015	A machine learning-based model developed using dipeptide deviation from expected mean.	69.12	0.754	0.386	73	65.49	ABCpred data set, Chen data set, Bcpred data set, SVMTrip data set, LBTope data set, EPMLR data set

TABLE 4 (Continued)

Name	Year	Description	Performance measure					Data set
			Acc	AUC	MCC	Spec	Sens	
DMN-LBE (Lian et al., 2015)	2015	A sequence-based predictor developed using deep maxout network with dropout training techniques.	68.33%	0.743	0.37	67.94	68.72	LFNR[b] data set
DRREP (Lian et al., 2015)	2017	A deep learning-based predictor for B-cell epitopes.	86.00%	0.862	-	0.75	-	SARS data set, Pellequer, HIV data set, AntiJen, SEQ194 data set
iBCE-EL (Manavalan et al., 2018)	2018	B-cell epitope prediction using ensemble method, combining extremely randomized tree and gradient boosting classifiers.	0.732	0.789	0.463	0.724	0.742	iBCE-EL data set
DLBEpitope (Liu et al., 2020)	2020	A deep learning-based predictor that combines existing methods.	-	0.957	-	-	-	DLBEpitope data set, LF,[a] ABCpred16
BCEPS (Ras-Carmona et al., 2021)	2021	An attempt has been made to predict B-cell epitopes that can induce cross-reactive antibodies with native antigens.	75.38%		0.51	0.78	0.73	BCEPS data set, abYbank/AbDb
LBCEPred (Alghamdi et al., 2022)	2022	A random forest model developed using statistical moment-based descriptors to predict the B-cell epitopes.	0.868	0.934	-	-	-	-
Epidope (Collatz et al., 2020)	2021	A deep neural network to detect linear B-cell epitope regions on individual protein sequences.	-	0.67	-	-	-	-
BepiPred-3.0 (Clifford et al., 2022)	2022	It is an improved version of series BepiPred, developed using protein language models.	0.688	0.762	0.309	-	-	BepiPred-3.0 data set

[a]LF: Lbtope_Fixed data set.
[b]LFNR: Lbtope_Fixed_non_redundant.
[c]LV: Lbtope_Variable.
[d]LVNR: Lbtope_Variable_non_redundant.
[e]LC: Lbtope_Confirm.

researchers used ML techniques for developing prediction models. In 2006, Saha and Raghava proposed a neural network-based method ABCPred, for the prediction of linear BCEs. Similarly, Söllner and Mayer (2006) developed a method by combining the decision tree and nearest neighbor with the molecular operating environment (Wang & Pai, 2014). Several tools are reported in the literature that use ML approaches for BCE prediction (listed in Table 4). The LBtope method developed by Singh et al. (2013) uses experimentally validated non-BCEs for the first time to predict the continuous BCEs using support vector machine (SVM) and K-nearest neighbor algorithms. Afterward, many deep learning-based methods have been developed.

5.3 | Miscellaneous methods

The ability to determine whether a particular sequence is a linear epitope has increased over the past few years; however, these techniques are unable to distinguish between IgG, IgE, or IgA epitopes. There are just a few

methods, including AlgPred, AlgPred2, and BCIgEpred, that can predict the subtype of an epitope (Saha & Raghava, 2006; Sharma et al., 2021; Saravanam & Gautham, 2018). For the aim of predicting BCEs that potentially induce a specific class of antibody, Gupta et al. (2013) developed the IgPred approach (Gupta et al., 2013; Tung et al., 2021). Since there is currently no comprehensive approach for predicting class-specific BCEs, with the exception of a few methods developed for IgE-producing allergenic epitopes, IgPred was the first tool created to predict specific BCEs (Gupta et al., 2013). EpiPred, a method proposed in 2014 by Krawczyk et al., 2014 that uses a docking-like approach to match up antibody and antigen structures in order to find epitope areas/regions on the antigen. Similarly, more approaches of these types have been listed in Table 5.

6 | DISCUSSION AND CHALLENGES

Ag–Ab interactions play a vital role in immune responses, particularly in the elimination of invading pathogens. Existing methods have shown that more than 90% of BCEs are conformational, while only 10% are linear BCEs (Sanchez-Trincado et al., 2017). Although various tools and methods are available for BCE prediction, their utility in designing vaccine candidates is currently limited. However, understanding BCEs can assist in the diagnosis and treatment of autoimmune diseases. It can also aid in the development of targeted therapies to suppress immune responses triggered by self-antigens. In this study, we provide a comprehensive overview of experimental methods, available databases/repositories of BCEs, and in silico prediction tools.

Additionally, we discuss new approaches in antibody-based epitope prediction. While several experimental approaches exist, they can be expensive in terms of cost, time, and effort required. Furthermore, the accuracy of computational tools heavily relies on the quality of data collected from publicly available repositories. This has led to an increase in redundant sequences and ambiguity in databases such as IEDB, where multiple sequences are assigned as both BCEs and non-BCEs, as highlighted in a study by Singh et al., 2013b. This ambiguity poses a challenge in identifying

TABLE 5 Other available methods.

Name	Year	Description	Performance measure					Data set
			Acc	AUC	MCC	Spec	Sens	
IgPred (Gupta et al., 2013)	2013	First method developed for predicting class-specific epitopes, like IgE, IgA, and IgG epitopes.	70.4[a], 82.7[b], 72.07[c]	0.76[a], 0.88[b], 0.78[c]	0.41[a], 0.66[b], 0.44[c]	-	-	IgPred data set
EPIPRED (Krawczyk et al., 2014)	2014	Docking-based approach for predicting epitope in an antigen based on its binding with antibodies.	-	-	-	-	-	SAbDAb, EPIPRED data set
Lyra (Klausen et al., 2015)	2015	An automated method for building of B- and T-cell receptor structural models from their amino acid sequence.	-	-	-	-	-	IMGT/3Dstructure-DB
PEASE (Sela-Culang et al., 2014)	2015	A method predict B-cell epitope for a given antibody sequence.	-	-	-	-	-	PEASE data set
NIgPred (Tung et al., 2021)	2021	It allows to predict IgA-, IgG-, and IgE-specific epitopes.	87.2,[a] 81.9,[b] 85.7[c]	-	74.5,[a] 64.0,[b] 71.5[c]	-	-	NIgPred data set, ADFS, Allerbase
AbCPE (Kadam et al., 2021)	2021	A method for predicting epitopes for specific class of antibodies that include IgA, IgG, IgE, and IgM.	-	-	-	-	-	AbCPE data set, SARS-CoV-2 data set

Abbreviations: ADFS, Allergen Database for Food Safety; SAbDAb, structural antibody database.
[a]IgG.
[b]IgE.
[c]IgA.

correct BCEs despite being generated through experimental methods. In recent years, significant progress has been made in the development of in silico tools for epitope prediction. These tools claim to outperform other methods; however, developing a method that performs consistently well on all types of data sets remains a challenge. The introduction of new ML and DL methods, such as AlphaFold, (Jumper et al., 2021) has revolutionized protein structure prediction by achieving atomic-level precision. This breakthrough has inspired researchers to design or develop accurate BCE prediction methods by leveraging predicted protein structures and pre-trained language models. It is generally easier to predict linear BCEs compared to conformational BCEs, as the latter relies on patterns within the surrounding biological environment and requires knowledge of the structure of the Ag–Ab complex, which is experimentally challenging. Although discontinuous epitopes have been identified, their utility as vaccine candidates is limited due to the loss of pattern or structure in a synthetic environment. They are primarily used as biomarkers in epitope mapping. The advancement of next-generation sequencing techniques, providing accurate and complete pathogenic genome sequences, has contributed to the development of advanced methods for epitope prediction. Immunoinformatic methods for epitope prediction and subsequent development of peptide-based vaccines have reduced costs, time, and increased accuracy compared to traditional laboratory tests (Raoufi et al., 2020). However, the precise determination of epitope characteristics that allow biorecognition of the Ag–Ab complex is still challenging (Potocnakova et al., 2016).

AUTHOR CONTRIBUTIONS

Nishant Kumar: Methodology; writing—review and editing; writing—original draft. **Nisha Bajiya**: Methodology; writing—review & editing; writing—original draft. **Sumeet Patiyal**: Methodology; writing—review and editing; writing—original draft. **Gajendra P. S. Raghava**: Conceptualization; investigation; funding acquisition; validation; visualization; writing—review and editing; writing—original draft; supervision; resources; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Abbott WM, Damschroder MM, Lowe DC. Current approaches to fine mapping of antigen-antibody interactions. Immunology. 2014;142:526–35.

Ahmad TA, Eweida AE, Sheweita SA. B-cell epitope mapping for the design of vaccines and effective diagnostics. Trials Vaccinol. 2016;5:71–83.

Alghamdi W, Attique M, Alzahrani E, Ullah MZ, Khan YD. LBCEPred: a machine learning model to predict linear B-cell epitopes. Brief Bioinform. 2022;23(3). https://doi.org/10.1093/bib/bbac035

Alix AJP. Predictive estimation of protein linear epitopes by using the program PEOPLE. Vaccine. 1999;18(3–4):311–4. https://doi.org/10.1016/s0264-410x(99)00329-1

Amit AG, Mariuzza RA, Phillips SE, Poljak RJ. Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. Science. 1986;233:747–53.

Ansari HR, Raghava GP. Identification of conformational B-cell epitopes in an antigen from its primary sequence. Immunome Res. 2010;6:6.

Arnold BF, Scobie HM, Priest JW, Lammie PJ. Integrated serologic surveillance of population immunity and disease transmission. Emerg Infect Dis. 2018;24:1188–94.

Ashford J, Reis-Cunha J, Lobo I, Lobo F, Campelo F. Organism-specific training improves performance of linear B-cell epitope prediction. Bioinformatics. 2021;37:4826–34.

Atassi MZ, Smith JA. A proposal for the nomenclature of antigenic sites in peptides and proteins. Immunochemistry. 1978;15:609–10.

Baerga-Ortiz A, Hughes CA, Mandell JG, Komives EA. Epitope mapping of a monoclonal antibody against human thrombin by H/D-exchange mass spectrometry reveals selection of a diverse sequence in a highly conserved protein. Protein Sci. 2002;11:1300–8.

Bannwarth L, Girerd-Chambaz Y, Arteni A, Guigner J-M, Ronzon F, Manin C, et al. Mapping of the epitopes of poliovirus type 2 in complex with antibodies. Mol Immunol. 2015;67:233–9.

Benjamin DC, Berzofsky JA, East IJ, Gurd FR, Hannum C, Leach SJ, et al. The antigenic structure of proteins: a reappraisal. Annu Rev Immunol. 1984;2:67–101.

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res. 2000;28:235–42.

Blythe MJ, Flower DR. Benchmarking B cell epitope prediction: underperformance of existing methods. Protein Sci. 2005;14: 246–8.

Caliandro R, Belviso DB, Aresta BM, de Candia M, Altomare CD. Protein crystallography and fragment-based drug design. Future Med Chem. 2013;5:1121–40.

Chaplin DD. Overview of the immune response. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S3–S23. https://doi.org/10.1016/j.jaci.2009.12.980

Clifford JN, Høie MH, Deleuran S, Peters B, Nielsen M, Marcatili P. BepiPred-3.0: improved B-cell epitope prediction using protein language models. Protein Sci. 2022;31(12). https://doi.org/10.1002/pro.4497

Coales SJ, Tuske SJ, Tomasso JC, Hamuro Y. Epitope mapping by amide hydrogen/deuterium exchange coupled with immobilization of antibody, on-line proteolysis, liquid chromatography and mass spectrometry. Rapid Commun Mass Spectrom. 2009; 23:639–47.

Collatz M, Mock F, Barth E, Hölzer M, Sachse K, Marz M. EpiDope: a deep neural network for linear B-cell epitope prediction. Bioinformatics. 2020;37(4):448–55. https://doi.org/10.1093/bioinformatics/btaa773

Dalkas GA, Rooman M. SEPIa, a knowledge-driven algorithm for predicting conformational B-cell epitopes from the amino acid sequence. BMC Bioinform. 2017;18:95.

Davydov II, Tonevitskiĭ AG. Linear B-cell epitope prediction. Mol Biol. 2009;43(1):166–74. https://doi.org/10.1134/s0026893309010221 (Russian).

De Groot AS, Bosma A, Chinai N, Frost J, Jesdale BM, Gonzalez MA, et al. From genome to vaccine: in silico predictions, ex vivo verification. Vaccine. 2001;19:4385–95.

El-Manzalawy Y, Dobbs D, Honavar V. Predicting flexible length linear B-cell epitopes. Comput Syst Bioinformatics Conf. 2008; 7:121–32.

El-Manzalawy Y, Dobbs D, Honavar V. Predicting linear B-cell epitopes using string kernels. J Mol Recognit. 2008;21:243–55.

El-Manzalawy Y, Honavar V. Recent advances in B-cell epitope prediction methods. Immunome Res. 2010;6(Suppl 2):2.

Emini EA, Hughes JV, Perlow DS, Boger J. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J Virol. 1985;55(3):836–9. https://doi.org/10.1128/jvi.55.3.836-839.1985

Ferdous S, Kelm S, Baker TS, Shi J, Martin ACR. B-cell epitopes: discontinuity and conformational analysis. Mol Immunol. 2019;114:643–50.

Fivash M, Towler EM, Fisher RJ. BIAcore for macromolecular interaction. Curr Opin Biotechnol. 1998;9:97–101.

Frank R. The SPOT-synthesis technique. Synthetic peptide arrays on membrane supports—principles and applications. J Immunol Methods. 2002;267:13–26.

Galanis KA, Nastou KC, Papandreou NC, Petichakis GN, Pigis DG, Iconomidou VA. Linear B-cell epitope prediction for in silico vaccine design: a performance review of methods available via command-line interface. Int J Mol Sci. 2021;22:3210.

Gao J, Faraggi E, Zhou Y, Ruan J, Kurgan L. BEST: improved prediction of B-cell epitopes from antigen sequences. PLoS One. 2012;7(6):e40104. https://doi.org/10.1371/journal.pone.0040104

Garnier J, Osguthorpe DJ, Robson B. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J Mol Biol. 1978;120:97–120.

Gershoni JM, Roitburd-Berman A, Siman-Tov DD, Tarnovitski Freund N, Weiss Y. Epitope mapping: the first step in developing epitope-based vaccines. BioDrugs. 2007;21:145–56.

Giacò L, Amicosante M, Fraziano M, Gherardini PF, Ausiello G, Helmer-Citterich M, et al. B-Pred, a structure based B-cell epitopes prediction server. Adv Appl Bioinform Chem. 2012;5:11–21.

Gupta S, Ansari HR, Gautam A, Raghava GP. Identification of B-cell epitopes in an antigen for inducing specific class of antibodies. Biol Direct. 2013;8(1). https://doi.org/10.1186/1745-6150-8-27

Gupta S, Ansari HR, Gautam A, Open Source Drug Discovery Consortium, Raghava GPS. Identification of B-cell epitopes in an antigen for inducing specific class of antibodies. Biol Direct. 2013;8:27.

Halperin I, Ma B, Wolfson H, Nussinov R. Principles of docking: an overview of search algorithms and a guide to scoring functions. Proteins. 2002;47:409–43.

Haste Andersen P, Nielsen M, Lund O. Prediction of residues in discontinuous B-cell epitopes using protein 3D structures. Protein Sci. 2006;15:2558–67.

Hensen SMM, Derksen M, Pruijn GJM. Multiplex peptide-based B cell epitope mapping. Methods Mol Biol. 2014;1184:295–308.

Hopp TP, Woods KR. Prediction of protein antigenic determinants from amino acid sequences. Proc Natl Acad Sci U S A. 1981;78: 3824–8.

Huang J, Honda W. CED: a conformational epitope database. BMC Immunol. 2006;7:7.

Ivanciuc O, Schein CH, Braun W. SDAP: database and computational tools for allergenic proteins. Nucleic Acids Res. 2003;31: 359–62.

Janin J, Wodak S, Levitt M, Maigret B. Conformation of amino acid side-chains in proteins. J Mol Biol. 1978;125(3):357–86. https://doi.org/10.1016/0022-2836(78)90408-4

Jespersen MC, Mahajan S, Peters B, Nielsen M, Marcatili P. Antibody specific B-cell epitope predictions: leveraging information from antibody-antigen protein complexes. Front Immunol. 2019;10:298.

Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res. 2017;45:W24–9.

Jones S, Thornton JM. Prediction of protein-protein interaction sites using patch analysis. J Mol Biol. 1997;272:133–43.

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596:583–9.

Kadam K, Peerzada N, Karbhal R, Sawant S, Valadi J, Kulkarni-Kale U. Antibody Class(es) Predictor for Epitopes (AbCPE): a multi-label classification algorithm. Front Bioinform. 2021;1.

Kam Y-W, Lee WWL, Simarmata D, Harjanto S, Teng T-S, Tolou H, et al. Longitudinal analysis of the human antibody response to Chikungunya virus infection: implications for serodiagnosis and vaccine development. J Virol. 2012;86:13005–15.

Karplus PA, Schulz GE. Prediction of chain flexibility in proteins. Sci Nat. 1985;72:212–3.

Klausen MS, Anderson MV, Jespersen MC, Nielsen M, Marcatili P. LYRA, a webserver for lymphocyte receptor structural modeling. Nucleic Acids Res. 2015;43(W1):W349–55.

Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett. 1990; 276(1–2):172–4. https://doi.org/10.1016/0014-5793(90)80535-q

Kolaskar AS, Kulkarni-Kale U. Prediction of three-dimensional structure and mapping of conformational epitopes of envelope glycoprotein of Japanese encephalitis virus. Virology. 1999; 261:31–42.

Kozlova EEG, Cerf L, Schneider FS, Viart BT, NGuyen C, Steiner BT, et al. Computational B-cell epitope identification and production of neutralizing murine antibodies against Atroxlysin-I. Sci Rep. 2018;8:14904.

Krawczyk K, Liu X, Baker T, Shi J, Deane CM. Improving B-cell epitope prediction and its application to global antibody-antigen docking. Bioinformatics. 2014;30(16):2288–94. https://doi.org/10.1093/bioinformatics/btu190

Krawczyk K, Liu X, Baker T, Shi J, Deane CM. Improving B-cell epitope prediction and its application to global antibody-antigen docking. Bioinformatics. 2014;30:2288–94.

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): e1002829. https://doi.org/10.1371/journal.pcbi.1002829

Kringelum JV, Nielsen M, Padkjær SB, Lund O. Structural analysis of B-cell epitopes in antibody:protein complexes. Mol Immunol. 2013;53:24–34.

Kulkarni-Kale U, Bhosle S, Kolaskar AS. CEP: a conformational epitope prediction server. Nucleic Acids Res. 2005;33:W168–71.

Larsen JEP, Lund O, Nielsen M. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006;2:2.

Lee H, Brendle SA, Bywaters SM, Guan J, Ashley RE, Yoder JD, et al. A cryo-electron microscopy study identifies the complete H16.V5 epitope and reveals global conformational changes initiated by binding of the neutralizing antibody fragment. J Virol. 2015;89:1428–38.

Lee JH, Leaman DP, Kim AS, Torrents de la Peña A, Sliepen K, Yasmeen A, et al. Antibodies to a conformational epitope on gp41 neutralize HIV-1 by destabilizing the Env spike. Nat Commun. 2015;6:8167.

Levitt M. Conformational preferences of amino acids in globular proteins. Biochemistry. 1978;17(20):4277–85. https://doi.org/10.1021/bi00613a026

Lian Y, Ge M, Pan X-M. EPMLR: sequence-based linear B-cell epitope prediction method using multiple linear regression. BMC Bioinformatics. 2014;15(1). https://doi.org/10.1186/s12859-014-0414-y

Lian Y, Huang ZC, Ge M, Pan XM. An improved method for predicting linear B-cell epitope using deep maxout networks. Biomed Environ Sci. 2015;28(6):460–3. https://doi.org/10.3967/bes2015.065

Liang S, Zheng D, Standley DM, Yao B, Zacharias M, Zhang C. EPSVR and EPMeta: prediction of antigenic epitopes using support vector regression and multiple server results. BMC Bioinformatics. 2010;11(1). https://doi.org/10.1186/1471-2105-11-381

Liang S, Zheng D, Yao B, Zhang C. EPCES and EPSVR: prediction of B-cell antigenic epitopes on protein surfaces with conformational information. Immunoinformatics. 2020;289–97. https://doi.org/10.1007/978-1-0716-0389-5_16

Liu T, Shi K, Li W. Deep learning methods improve linear B-cell epitope prediction. BioData Mining. 2020;13(1). https://doi.org/10.1186/s13040-020-00211-0

Liu J, Zhang W. Databases for B-cell epitopes. Methods Mol Biol. 2014;1184:135–48.

Lo Conte L, Chothia C, Janin J. The atomic structure of protein-protein recognition sites. J Mol Biol. 1999;285:2177–98.

Lo Y-T, Shih T-C, Pai T-W, Ho L-P, Wu J-L, Chou H-Y. Conformational epitope matching and prediction based on protein surface spiral features. BMC Genomics. 2021;22:116.

Malito E, Faleri A, Lo Surdo P, Veggi D, Maruggi G, Grassi E, et al. Defining a protective epitope on factor H binding protein, a key meningococcal virulence factor and vaccine antigen. Proc Natl Acad Sci U S A. 2013;110:3304–9.

Manavalan B, Govindaraj RG, Shin TH, Kim MO, Lee G. IBCE-EL: a new ensemble learning framework for improved linear B-cell Epitope prediction. Front Immunol. 2018;9:1695.

Mayer M, Meyer B. Group epitope mapping by saturation transfer difference NMR to identify segments of a ligand in direct contact with a protein receptor. J Am Chem Soc. 2001;123:6108–17.

Moreau V, Granier C, Villard S, Laune D, Molina F. Discontinuous epitope prediction based on mimotope analysis. Bioinformatics. 2006;22:1088–95.

Morris GE. Epitope mapping: B-cell epitopes. eLS [Internet]. 2007. https://doi.org/10.1002/9780470015902.a0002624.pub2

Najar TA, Khare S, Pandey R, Gupta SK, Varadarajan R. Mapping protein binding sites and conformational epitopes using cysteine labeling and yeast surface display. Structure. 2017;25: 395–406.

Nilvebrant J, Rockberg J. An introduction to epitope mapping. Methods Mol Biol. 2018;1785:1–10.

Obungu VH, Gelfanova V, Rathnachalam R, Bailey A, Sloan-Lancaster J, Huang L. Determination of the mechanism of action of anti-FasL antibody by epitope mapping and homology modeling. Biochemistry. 2009;48:7251–60.

Odorico M, Pellequer J-L. BEPITOPE: predicting the location of continuous epitopes and patterns in proteins. J Mol Recognit. 2003;16(1):20–2. https://doi.org/10.1002/jmr.602

Olsen LR, Zhang GL, Reinherz EL, Brusic V (2011) FLAVIdB: a data mining system for knowledge discovery in flaviviruses with direct applications in immunology and vaccinology. Immunome Res. 7, 2. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25544857

Opuni KFM, Al-Majdoub M, Yefremova Y, El-Kased RF, Koy C, Glocker MO. Mass spectrometric epitope mapping. Mass Spectrom Rev. 2018;37:229–41.

Pandit D, Tuske SJ, Coales SJ, SY E, Liu A, Lee JE, et al. Mapping of discontinuous conformational epitopes by amide hydrogen/deuterium exchange mass spectrometry and computational docking. J Mol Recognit. 2012;25:114–24.

Parker JM, Guo D, Hodges RS. 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. 1986;25: 5425–32.

Patching SG. Surface plasmon resonance spectroscopy for characterisation of membrane protein-ligand interactions and its potential for drug discovery. Biochim Biophys Acta. 2014;1838:43–55.

Pellequer JL, Westhof E, Van Regenmortel MH. Correlation between the location of antigenic sites and the prediction of turns in proteins. Immunol Lett. 1993;36:83–99.

Pellequer JL, Westhof E, Van Regenmortel MH. Predicting location of continuous epitopes in proteins from their primary structures. Methods Enzymol. 1991;203:176–201.

Pellequer JL, Westhof E. PREDITOP: a program for antigenicity prediction. J Mol Graph. 1993;11(204–10):191–2.

Peters B, Sidney J, Bourne P, Bui H-H, Buus S, Doh G, et al. The immune epitope database and analysis resource: from vision to blueprint. PLoS Biol. 2005;3:e91.

Ponnuswamy PK, Prabhakaran M, Manavalan P. Hydrophobic packing and spatial arrangement of amino acid residues in globular proteins. Biochim Biophys Acta. 1980;623(2):301–16. https://doi.org/10.1016/0005-2795(80)90258-5

Ponomarenko J, Bui H-H, Li W, Fusseder N, Bourne PE, Sette A, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. BMC Bioinform. 2008;9:514.

Ponomarenko J, Van Regenmortel M. *Structural bioinformatics: B cell epitope prediction*. 2009.

Ponomarenko JV, Bourne PE. Antibody-protein interactions: benchmark datasets and prediction tools evaluation. BMC Struct Biol. 2007;7:64.

Ponomarenko JV, Marc HV. *35 B-cell epitope prediction*. 2008.

Potocnakova L, Bhide M, Pulzova LB. An introduction to B-cell epitope mapping and in silico epitope prediction. J Immunol Res. 2016;2016:6760830.

Qi T, Qiu T, Zhang Q, Tang K, Fan Y, Qiu J, et al. SEPPA 2.0—more refined server to predict spatial epitope considering species of immune host and subcellular localization of protein antigen. Nucleic Acids Res. 2014;42:W59–63.

Raoufi E, Hemmati M, Eftekhari S, Khaksaran K, Mahmodi Z, Farajollahi MM, et al. Epitope prediction by novel immunoinformatics approach: a state-of-the-art review. Int J Pept Res Ther. 2020;26:1155–63.

Ras-Carmona A, Pelaez-Prestel HF, Lafuente EM, Reche PA. BCEPS: a web server to predict linear B cell epitopes with enhanced immunogenicity and cross-reactivity. Cell. 2021;10(10):2744. https://doi.org/10.3390/cells10102744

Rubinstein ND, Mayrose I, Martz E, Pupko T. Epitopia: a web-server for predicting B-cell epitopes. BMC Bioinform. 2009;10:287.

Rueckert C, Guzmán CA. Vaccines: from empirical development to rational design. PLoS Pathog. 2012;8:e1003001.

Russi RC, Bourdin E, García MI, Veaute CMI. In silico prediction of T- and B-cell epitopes in PmpD: first step towards to the design of a *Chlamydia trachomatis* vaccine. Biomed J. 2018;41:109–17.

Rux JJ, Burnett RM. Type-specific epitope locations revealed by X-ray crystallographic study of adenovirus type 5 hexon. Mol Ther. 2000;1:18–30.

Söllner J, Mayer B. Machine learning approaches for prediction of linear B-cell epitopes on proteins. J Mol Recognit. 2006;19:200–8.

Saha S, Raghava GPS. BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties. Artificial Immune Systems. 2004;197–204. https://doi.org/10.1007/978-3-540-30220-9_16

Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins. 2006;65(1):40–8. https://doi.org/10.1002/prot.21078

Saha S, Bhasin M, Raghava GPS. Bcipep: a database of B-cell epitopes. BMC Genomics. 2005;6:79.

Saha S, Raghava GPS. AlgPred: prediction of allergenic proteins and mapping of IgE epitopes. Nucleic Acids Res. 2006;34:W202–9.

Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T- and B-cell epitope prediction. J Immunol Res. 2017;2017:2680160.

Santona A, Carta F, Fraghí P, Turrini F. Mapping antigenic sites of an immunodominant surface lipoprotein of *Mycoplasma agalactiae*, AvgC, with the use of synthetic peptides. Infect Immun. 2002;70:171–6.

Saravanan V, Gautham N. Harnessing computational biology for exact linear B-cell epitope prediction: a novel amino acid composition-based feature descriptor. OMICS. 2015;19(10):648–58. https://doi.org/10.1089/omi.2015.0095

Saravanan V, Gautham N. BCIgEPRED—a dual-layer approach for predicting linear IgE epitopes. Mol Biol. 2018;52:333–43.

Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum. 2000;43:155–63.

Schlessinger A, Ofran Y, Yachdav G, Rost B. Epitome: database of structure-inferred antigenic epitopes. Nucleic Acids Res. 2006;34:D777–80.

Schneider FS, Nguyen DL, Castro KL, Cobo S, Machado de Avila RA, Ferreira N d A, et al. Use of a synthetic biosensor for neutralizing activity-biased selection of monoclonal antibodies against atroxlysin-I, an hemorrhagic metalloproteinase from Bothrops atrox snake venom. PLoS Negl Trop Dis. 2014;8:e2826.

Sela-Culang I, Ashkenazi S, Peters B, Ofran Y. PEASE: predicting B-cell epitopes utilizing antibody sequence. Bioinformatics. 2014;31(8):1313–5. https://doi.org/10.1093/bioinformatics/btu790

Sharma N, Patiyal S, Dhall A, Pande A, Arora C, Raghava GPS. AlgPred 2.0: an improved method for predicting allergenic proteins and mapping of IgE epitopes. Brief Bioinform. 2021;22:bbaa294. https://doi.org/10.1093/bib/bbaa294

Sharma OP, Das AA, R K, Kumar MS, Mathur PP. Structural epitope database (SEDB): a web-based database for the epitope, and its intermolecular interaction along with the tertiary structure information. J Proteomics Bioinform. 2012;5:1-6. https://doi.org/10.4172/jpb.1000217

Sharon J, Rynkiewicz MJ, Lu Z, Yang C-Y. Discovery of protective B-cell epitopes for development of antimicrobial vaccines and antibody therapeutics. Immunology. 2014;142:1–23.

Shashkova TI, Umerenkov D, Salnikov M, Strashnov PV, Konstantinova AV, Lebed I, et al. SEMA: antigen B-cell conformational epitope prediction using deep transfer learning. Front Immunol. 2022;13. https://doi.org/10.3389/fimmu.2022.960985

Sher G, Zhi D, Zhang S. DRREP: deep ridge regressed epitope predictor. BMC Genomics. 2017;18(Suppl 6):676. https://doi.org/10.1186/s12864-017-4024-8

da Silva BM, Myung Y, Ascher DB, Pires DEV. epitope3D: a machine learning method for conformational B-cell epitope prediction. Brief Bioinform. 2021;23(1).

Siman-Tov DD, Zemel R, Tur Kaspa R, Gershoni JM. The use of epitope arrays in immunodiagnosis of infectious disease: hepatitis C virus, a case study. Anal Biochem. 2013;432:63–70.

Singh H, Gupta S, Gautam A, Raghava GPS. Designing B-cell epitopes for immunotherapy and subunit vaccines. Peptide Antibodies. 2015;327–40. https://doi.org/10.1007/978-1-4939-2999-3_28

Singh H, Ansari HR, Raghava GPS. Improved method for linear B-cell epitope prediction using antigen's primary sequence. PLoS One. 2013;8:e62216.

Solihah B, Azhari A, Musdholifah A. Enhancement of conformational B-cell epitope prediction using CluSMOTE. PeerJ Comput Sci. 2020;6:e275.

Suckau D, Köhl J, Karwath G, Schneider K, Casaretto M, Bitter-Suermann D, et al. Molecular epitope identification by limited proteolysis of an immobilized antigen-antibody complex and mass spectrometric peptide mapping. Proc Natl Acad Sci U S A. 1990;87:9848–52.

Sun H, Ma L, Wang L, Xiao P, Li H, Zhou M, et al. Research advances in hydrogen-deuterium exchange mass spectrometry for protein epitope mapping. Anal Bioanal Chem. 2021;413:2345–59.

Sun J, Wu D, Xu T, Wang X, Xu X, Tao L, et al. SEPPA: a computational server for spatial epitope prediction of protein antigens. Nucleic Acids Res. 2009;37:W612–6.

Sun P, Chen W, Huang Y, Wang H, Ma Z, Lv Y. Epitope prediction based on random peptide library screening: benchmark dataset and prediction tools evaluation. Molecules. 2011;16:4971–93.

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.

Sweredoski MJ, Baldi P. COBEpro: a novel system for predicting continuous B-cell epitopes. Protein Eng Des Sel. 2008;22(3):113–20. https://doi.org/10.1093/protein/gzn075

Sweredoski MJ, Baldi P. PEPITO: improved discontinuous B-cell epitope prediction using multiple distance thresholds and half sphere exposure. Bioinformatics. 2008;24:1459–60.

Temporini C, Bavaro T, Tengattini S, Serra I, Marrubini G, Calleri E, et al. Liquid chromatography-mass spectrometry structural characterization of neo glycoproteins aiding the rational design and synthesis of a novel glycovaccine for protection against tuberculosis. J Chromatogr A. 2014;1367:57–67.

Tomar N, De RK. A brief outline of the immune system. Methods Mol Biol. 2014;1184:3–12.

Toseland CP, Clayton DJ, McSparron H, Hemsley SL, Blythe MJ, Paine K, et al. AntiJen: a quantitative immunology database integrating functional, thermodynamic, kinetic, biophysical, and cellular data. Immunome Res. 2005;1:4.

Tung C-H, Chang Y-S, Chang K-P, Chu Y-W. NIgPred: class-specific antibody prediction for linear B-cell epitopes based on heterogeneous features and machine-learning approaches. Viruses. 2021;13:1531.

Van Regenmortel MHV. Immunoinformatics may lead to a reappraisal of the nature of B cell epitopes and of the feasibility of synthetic peptide vaccines. J Mol Recognit. 2006;19:183–7.

Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, et al. The Immune Epitope Database (IEDB): 2018 update. Nucleic Acids Res. 2019;47:D339–43.

Wang H-W, Lin Y-C, Pai T-W, Chang H-T. Prediction of B-cell linear epitopes with a combination of support vector machine classification and amino acid propensity identification. J Biomed Biotechnol. 2011;2011:1–12. https://doi.org/10.1155/2011/432830

Wang Y, Wu W, Negre NN, White KP, Li C, Shah PK. Determinants of antigenicity and specificity in immune response for protein sequences. BMC Bioinformatics. 2011;12(1). https://doi.org/10.1186/1471-2105-12-251

Wang H-W, Pai T-W. Machine learning-based methods for prediction of linear B-cell epitopes. Methods Mol Biol. 2014;1184:217–36.

Wee LJ, Simarmata D, Kam Y-W, Ng LF, Tong JC. SVM-based prediction of linear B-cell epitopes using Bayes Feature Extraction. BMC Genomics. 2010;11(S4). https://doi.org/10.1186/1471-2164-11-s4-s21

Welling GW, Weijer WJ, van der Zee R, Welling-Wester S. Prediction of sequential antigenic regions in proteins. FEBS Lett. 1985;188:215–8.

Westhof E, Altschuh D, Moras D, Bloomer AC, Mondragon A, Klug A, et al. Correlation between segmental mobility and the location of antigenic determinants in proteins. Nature. 1984;311:123–6.

Wind T, Jensen MA, Andreasen PA. Epitope mapping for four monoclonal antibodies against human plasminogen activator inhibitor type-1: implications for antibody-mediated PAI-1-neutralization and vitronectin-binding. Eur J Biochem. 2001;268:1095–106.

Yang X, Yu X. An introduction to epitope prediction methods and software. Rev Med Virol. 2009;19:77–96.

Yao B, Zhang L, Liang S, Zhang C. SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate Tri-peptide similarity and propensity. PLoS One. 2012;7(9):e45152. https://doi.org/10.1371/journal.pone.0045152

Zander H, Reineke U, Schneider-Mergener J, Skerra A. Epitope mapping of the neuronal growth inhibitor Nogo-A for the Nogo receptor and the cognate monoclonal antibody IN-1 by means of the SPOT technique. J Mol Recognit. 2007;20:185–96.

Zhang J, Zhao X, Sun P, Gao B, Ma Z. Conformational B-cell epitopes prediction from sequences using cost-sensitive ensemble classifiers and spatial clustering. Biomed Res Int. 2014;2014:689219.

Zhang Q, Willison LN, Tripathi P, Sathe SK, Roux KH, Emmett MR, et al. Epitope mapping of a 95 kDa antigen in complex with antibody by solution-phase amide backbone hydrogen/deuterium exchange monitored by Fourier transform ion cyclotron resonance mass spectrometry. Anal Chem. 2011;83:7129–36.

Zhou C, Chen Z, Zhang L, Yan D, Mao T, Tang K, et al. SEPPA 3.0—enhanced spatial epitope prediction enabling glycoprotein antigens. Nucleic Acids Res. 2019;47(W1):W388–94. https://doi.org/10.1093/nar/gkz413

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