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# Identification of conformational B-cell Epitopes in an antigen from its primary sequence

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## Abstract

**Background:** One of the major challenges in the field of vaccine design is to predict conformational B-cell epitopes in an antigen. In the past, several methods have been developed for predicting conformational B-cell epitopes in an antigen from its tertiary structure. This is the first attempt in this area to predict conformational B-cell epitope in an antigen from its amino acid sequence.

**Results:** All Support vector machine (SVM) models were trained and tested on 187 non-redundant protein chains consisting of 2261 antibody interacting residues of B-cell epitopes. Models have been developed using binary profile of pattern (BPP) and physiochemical profile of patterns (PPP) and achieved a maximum MCC of 0.22 and 0.17 respectively. In this study, for the first time SVM model has been developed using composition profile of patterns (CPP) and achieved a maximum MCC of 0.73 with accuracy 86.59%. We compare our CPP based model with existing structure based methods and observed that our sequence based model is as good as structure based methods.

**Conclusion:** This study demonstrates that prediction of conformational B-cell epitope in an antigen is possible from its primary sequence. This study will be very useful in predicting conformational B-cell epitopes in antigens whose tertiary structures are not available. A web server CBTOPE has been developed for predicting B-cell epitope <http://www.imtech.res.in/raghava/cbtope/>.

## Background

A region or segment of an antigen, recognized by a specific antibody or B-cell is called antigenic region or B-cell epitope. These B-cell epitopes can be categorized into two classes, continuous and discontinuous. A continuous/linear epitope is a segment of consecutive residues in the primary sequence while a discontinuous/conformational epitope is a bunch of residues of an antigen that are far away from each other in the primary sequence but are brought to spatial proximity as a result of polypeptide folding. It is also known that most of the B-cell epitope (~90%) are conformational epitope. Both types of epitopes play an important role in the peptide-based vaccines and disease diagnosis [1,2]. One of the beauties of immune system is that it recognizes the foreign proteins/antigens and generate specific antibody against these antigens. This potential of immune system has been exploited by researchers for designing subunit vaccines [3,4].

In the post genomic era where a large number of pathogens have been completely sequenced, it is crucial to identify B-cell epitope or here after called antibody interacting residues in an antigen for the design of subunit vaccines against these pathogens. In the past several experimental techniques have been developed for mapping antibody interacting residues on an antigen that includes identification of interacting residues from structure of antibody-antigen complexes [5]. One of the popular approaches is overlapping peptide synthesis covering the entire antigen sequence, which identifies mainly sequential epitopes [6]. Mapping of antibody interacting residues has been severely hampered by the costly and time taking process of 3D structure determination. Many tools, covering compilation, visualization and prediction of B and T cell epitopes have been developed [7]. Despite of majority of epitopes being conformational, most of the computational methods and databases centered at the sequential epitopes [8-10]. Linear epitope prediction methods can be categorized into physico-chemical property [11], HMM [12] and

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ANN based [13]. Many methods are available for antibody interacting residues identification if antigen's or its homolog's tertiary structure is known which in itself is a big limitation. These are based on features like flexibility, solvent accessibility [14,15] and amino acid propensity scales [16]. Earlier researchers created a benchmark dataset from the 3D PDB structures and evaluated several structure-based protein-protein binding site prediction methods which included popular CEP [15] and DiscoTope [16] for predicting immunogenic regions [17]. They opted the definition, that epitope consist of antigen residues in which any atom of the antigen residue is separated from any antibody atom by a distance of  $\leq 4\text{\AA}$ . They found that the performance of all methods were mediocre and no method could achieve Area under curve (AUC) greater than 0.7. In addition to these a bunch of improved methods have been developed for the prediction of antibody interacting residues if tertiary structure of antigen is known [18-23]. In summary, one needs to determine structure of antigen using crystallography in order to identify antibody interacting residues in antigen. The experimental techniques like crystallography are expensive and time consuming where as functional assays are not reliable enough [5]. Thus there is need to develop alternate technique for predicting antibody interacting residues in a protein.

In this study attempt has been made to predict antibody interacting residues in an antigen from its primary sequence. First we created the patterns of different window lengths from the corresponding amino acid sequences then used the standard binary and physico-chemical profiles of patterns. We have introduced for the first time the concept of composition profile of pattern (CPP) generated through sliding window where the central residue is antibody interacting. These features were used to develop SVM based models to predict antibody interacting residues with high accuracy.

## Methods

### Definition of antibody interacting residues or epitope

There are many levels of antigen-antibody interactions one can obtain from PDB structures. Among these interactions we defined antibody interacting residue as a residue of antigen which is at least one atom separated from an antibody atom by  $4\text{\AA}$  distance. We borrowed this definition from benchmark paper [17] in order to compare our models with existing methods.

### Datasets

#### Main dataset

We obtained 526 antigenic sequences combined from IEDB database and benchmark dataset [9,17]. Sequence redundancy was removed using program CDHIT [24] at 40% cutoff. Finally we got 187 antigens where no two

sequences have more than 40% sequence identity. These antigens have 2261 antibody interacting or 2261 residues are part of conformational B-cell epitope and 107414 amino acid residues were non-antibody interacting from the same antigen sequences.

#### Benchmark Dataset

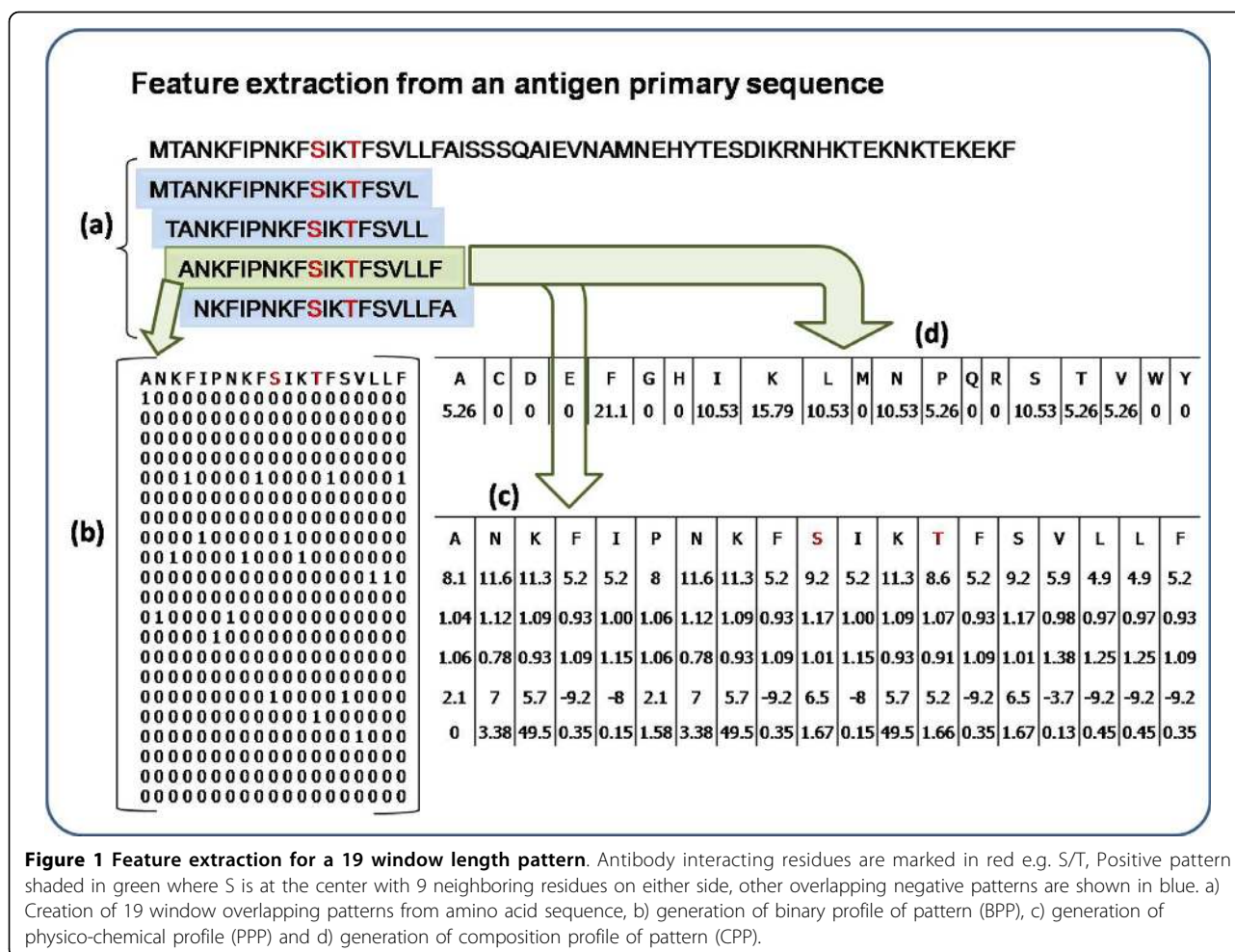
In addition to main dataset, we also evaluate our models on benchmark dataset [17] which contains 161 protein chains from 144 antigen-antibody complex structures. Finally we got non-redundant set of 52 antigen chains where no two sequences have more than 40% sequence identity. This benchmark dataset of 52 antigens contains 858 antibody interacting and 9366 non-antibody interacting residues.

#### Creation of patterns

It is known that the function of a residue is not solely determined by itself but influenced by its neighboring residues [25-27]. Thus we generated overlapping patterns of different window sizes from 5 to 21 amino acids for each antigen in the datasets. A pattern is assigned as positive if its central residue interacts with the antibody; else it is assigned as negative (Figure 1). This is the standard procedure used for assigning patterns, which have been used in number of methods like prediction of NAD interacting residues [26], DNA, RNA binding sites in proteins [27], cleavage sites [28] and signal peptides [29]. In order to create a pattern for the terminal residues, we added  $(L-1)/2$  number of dummy residue 'X' on both sides of the protein sequence (L is length of the protein sequence) for e.g. for window size 17 we added 8 'X'.

#### Realistic and balance learning

In order to develop prediction method one needs to generate overlapping patterns for each antigen in a dataset; one pattern for each residue. It will produce two types of patterns positive and negative, positive patterns have antibody interacting central residue. These patterns are used to train machine-learning techniques for developing models. In real life only few residues in an antigen are recognized by antibody or B-cell receptor. This means that the number of negative patterns will be much higher than positive patterns in our training dataset; for 2261 positive patterns there were 107414 negative patterns. This creates two problems; i) poor performance of models due to imbalanced set of patterns and ii) training of models is time consuming and CPU intensive. Thus in this study we have used two pattern sets for learning our models; i) realistic set of patterns that includes all negative patterns and ii) balance set of patterns having equal number of positive and negative patterns. In case of balance set, we randomly picked up equal number of negatives from negative pattern set.



**Figure 1** Feature extraction for a 19 window length pattern. Antibody interacting residues are marked in red e.g. S/T, Positive pattern shaded in green where S is at the center with 9 neighboring residues on either side, other overlapping negative patterns are shown in blue. a) Creation of 19 window overlapping patterns from amino acid sequence, b) generation of binary profile of pattern (BPP), c) generation of physico-chemical profile (PPP) and d) generation of composition profile of pattern (CPP).

#### Derivation of features from patterns

##### Binary profile of patterns (BPP)

Each pattern was converted into binary profile, where an amino acid was represented by a vector of dimension 21 (e.g. Ala by 1,0). A pattern of window length  $W$  was represented by a vector of dimensions  $21 \times W$  (Additional file 1, Table S1). The binary profile has been used in a number of existing methods [30,31].

##### Physico-chemical profile of patterns (PPP)

As amino acids' physico-chemical properties contribute in the determination of its structure and function, we selected five properties tested by others [32]. These are Grantham polarity [33], Karplus-Schulz flexibility [34], Kolaskar antigenicity [35], Parker hydrophobicity [36] and Ponnuswami polarity index [37]. Physico-chemical profile of patterns is similar to the BPP, the only difference lies in the properties of amino acids. Here each amino acid is represented by a vector of 5 i.e. each pattern converted into a vector size of  $5 \times W$ . For example Ala is represented as [pHydrophobicity, pFlexibility,

pPolarity\_Grantham, pPolarity\_Ponnuswami, pAntigenicity] corresponding to different property values (Additional file 1, Table S2).

##### Composition profile of patterns (CPP)

In the past researchers have exploited amino acid composition of proteins for many biological problems like sub-cellular localization and classification of proteins [38,39]. Instead of calculating composition of antigen sequence, we introduced concept of composition of patterns. The amino acid composition of patterns was calculated using the following equation.

$$comp(i) = \frac{R_i}{N} \times 100$$

Where  $comp(i)$  is the percent composition of a residue of type  $i$ ;  $R_i$  is number of residues of type  $i$ , and  $N$  is the total number of residues in the pattern.

##### Support Vector Machines (SVM)

In the past SVM had been used in a number of biological problems, from classification to functional prediction

of proteins [40-42]. In the present study, we have developed a SVM model using a powerful package SVM\_light <http://svmlight.joachims.org/>, for predicting antibody interacting residues in proteins.

#### Cross-validation technique

There are many techniques for evaluating the performance of models like leave-one-out or jack-knife test, n-fold cross validation etc [43]. Though jackknife test is the best among cross-validation techniques [44], it is time consuming and CPU intensive technique [40,45]. In order to save time and resources we used widely acceptable 5-fold cross-validation technique. In this technique data is randomly divided into five equal sets of which four sets are used for training and the remaining fifth set for testing. This process is repeated five times in such a way that each set is used once for testing. Final performance is the average of performances achieved on the five sets.

#### Performance Measures

The performance of various models developed in this study was computed by using threshold-dependent as well as threshold-independent parameters. In threshold-dependent parameters we used sensitivity (Sen), Specificity (Spe) or percent coverage of non-interacting residues, overall accuracy (Acc) and Matthew's correlation coefficient (MCC) using following equations.

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100$$

$$\text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \times 100$$

$$\text{MCC} = \frac{(TP \times TN) - (FP \times FN)}{\sqrt{[(TP + FN)(TN + FP)(TP + FP)(TN + FN)]}}$$

[TP = true positive; FN = false negative; TN = true negative; FP = false positive]

We created ROC (receiver operating curve) for all of the models in order to evaluate performance of models using threshold independent parameters. ROC plots with Area under curve (AUC) were created using SPSS statistical package.

## Results

### Analysis of antibody interacting residues

In order to understand whether certain types of amino acids are preferred in antibody interactions, we compared

the composition of antibody interacting and non-interacting residues in antigens. As shown in Figure 2, certain types of residues like Cysteine, Aspartate, Glutamate, Lysine, Asparagine, Glutamine, Arginine, Tryptophan and Tyrosine are preferred in antibody interactions. Most of these are polar and charged residues. In order to understand the preference of interaction in depth, we created 2 Sample Logos [46] for different properties. It was observed that charged, hydrophilic, surface exposed and flexible residues are more abundant in conformational B-cell epitopes (Additional file 1, Figures S1, S2, S3, S4, and S5).

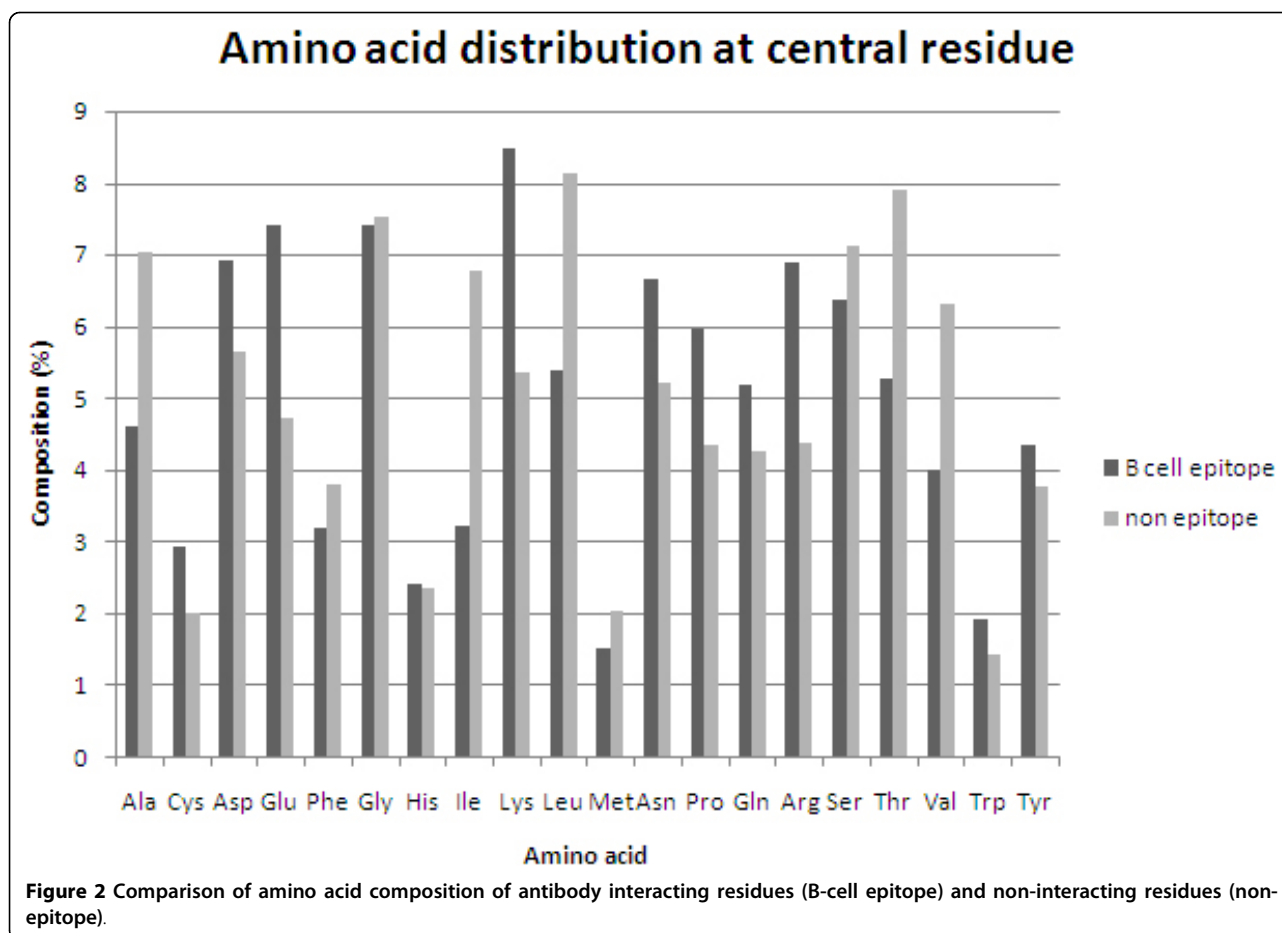
### SVM Models based on BPP and PPP

First, SVM based models have been developed using binary profile of patterns where pattern is represented by a vector of dimensions Nx21 (N is length of pattern). In order to optimize the performance of SVM models, we developed SVM models using patterns of window length 5 to 21. It was observed that models perform better for window size 13, where we got maximum MCC 0.22 with accuracy of 60.84% (Table1). We selected models with minimum difference between sensitivity and specificity. Varying the kernel parameters could not enhance the performance of models and results were just better than random. Detail performance of BPP based SVM model for window length 13 at different thresholds is shown in Additional file 1, Table S3.

It was observed that amino acids having certain types of physico-chemical properties are preferred in antibody interactions (Additional file 1, Figures S1, S2, S3, S4, and S5). Thus we developed SVM based models using PPP and observed best performance for pattern length of 15 residues. As shown in Table 2, we got maximum MCC 0.17 with accuracy 58.31%. The trend and performance of SVM models based on BPP and PPP is similar. Detail performance of PPP based SVM model for window length 15 at different thresholds is shown in Additional file 1, Table S4. Overall performance of PPP based model is slightly poorer than BPP based model (Additional file 1, Tables S3 and S4). All models were trained and tested on main dataset using balance set of patterns.

### SVM Model using Composition Profile of Patterns (CPP)

To understand the antibody interacting patterns better, we computed and compared amino acid composition of positive and negative patterns. As shown in Additional file 1, Figure S6, composition profile of positive and negative patterns are different. This means that positive and negative patterns can be discriminated from their amino acid composition. Based on this observation, we developed SVM models for predicting antibody interacting residues in proteins using composition profile of



patterns (CPP). The performance of CPP based SVM models have been shown in Table 3. It is surprising that simple composition based model outperforms BPP and CPP based models. We achieved maximum MCC 0.73 with accuracy 86.59% at window length 19. Detail performance of CPP based SVM model for window length 19 is shown in Additional file 1, Table S5. The

performance improved significantly for almost all window sizes as compared to binary or physico-chemical properties. As shown in Figure 3, we achieved area under curve (AUC) 0.90 which is significantly better than AUC achieved using BPP and PPP based models. All models were developed from main dataset using balance set of patterns and evaluated using five-fold cross-validation technique.

**Table 1** The performance of BPP based SVM model developed using different window lengths from 5 to 21 residues

Window size	Kernel parameters	Thr*	Sen	Spe	Acc	MCC
5	t 2 g 0.01 j 1 c 10	0.1	58.38	58.55	58.47	0.17
7	t 2 g 0.01 j 1 c 1	0.1	55.87	59.81	57.84	0.16
9	t 2 g 0.01 j 1 c 1	0.1	55.66	58.85	57.26	0.15
11	t 2 g 0.001 j 1 c 10	0	61.55	56.99	59.27	0.19
13	t 2 g 0.1 j 1 c 1	0	62.58	59.09	60.84	0.22
15	t 2 g 0.1 j 1 c 10	0	59.93	57.63	58.78	0.18
17	t 2 g 0.001 j 1 c 10	0	58.37	57.18	57.78	0.16
19	t 2 g 0.001 j 1 c 10	0.1	52.92	63.78	58.35	0.17
21	t 2 g 0.001 j 1 c 10	0	59.69	57.22	58.45	0.17

\*(Thr- Threshold, Sen - Sensitivity, Spe - Specificity, Acc - Accuracy, MCC - Matthew's correlation coefficient).

**Table 2** The performance of PPP based SVM model developed different window lengths from 5 to 21 residues

W	Kernel parameters	Thr*	Sen	Spe	Acc	MCC
5	t 2 g 0.00001 j 1 c 10	-0.3	53.95	59.62	56.78	0.14
7	t 2 g 0.00001 j 1 c 10	0.1	55.82	58.03	56.93	0.14
9	t 2 g 0.00001 j 1 c 10	0	54.56	55.84	55.2	0.1
11	t 2 g 0.00001 j 1 c 10	0.1	52.3	62.48	57.39	0.15
13	t 2 g 0.00001 j 1 c 10	0.1	55.11	60.37	57.74	0.16
15	t 2 g 0.00001 j 1 c 10	0	56.57	60.06	58.31	0.17
17	t 2 g 0.00001 j 1 c 10	0	60.19	55.77	57.98	0.16
19	t 2 g 0.00001 j 1 c 10	0	57.82	54.15	55.98	0.12
21	t 1 d 1	0	57.31	58.32	57.81	0.16

**Table 3 The performance SVM models developed using composition profile of patterns at different window lengths**

Window size	Kernel parameters	Thr*	Sen	Spe	Acc	MCC
5	t 2 g 0.001 j 1 c 1	0	61.75	58.11	59.93	0.2
7	t 2 g 0.001 j 1 c 10	0	68.35	62.2	65.27	0.31
9	t 2 g 0.001 j 1 c 10	0	73.45	67.21	70.33	0.41
11	t 2 g 0.01 j 1 c 1	-0.1	82.08	77.26	79.67	0.59
13	t 2 g 0.01 j 1 c 10	-0.1	82.57	84.17	83.37	0.67
15	t 2 g 0.01 j 1 c 1	-0.1	79.96	90.31	85.14	0.71
17	t 2 g 0.01 j 1 c 1	-0.1	80.69	90.1	85.4	0.71
19	<b>t 2 g 0.01 j 1 c 1</b>	<b>-0.1</b>	<b>83.13</b>	<b>90.06</b>	<b>86.59</b>	<b>0.73</b>
21	t 2 g 0.01 j 1 c 1	-0.1	83.62	88.96	86.29	0.73

### Comparison with existing methods

In order to validate our observations, we developed and evaluated our models on benchmark dataset; a dataset used in the past to benchmark earlier methods. All window size patterns were made unique and divided into realistic and balance set of patterns. Realistic set of patterns represents the real-life situation where non interacting residues are much higher than interacting residues. We trained and tested our models on benchmark dataset using balance set of patterns and achieved MCC 0.13 and 0.72 for BPP and CPP respectively (Table 4). These results demonstrates that CPP based models are also effective on benchmark dataset. In order to make evaluation more realistic, we also trained and tested our models using realistic set of patterns based

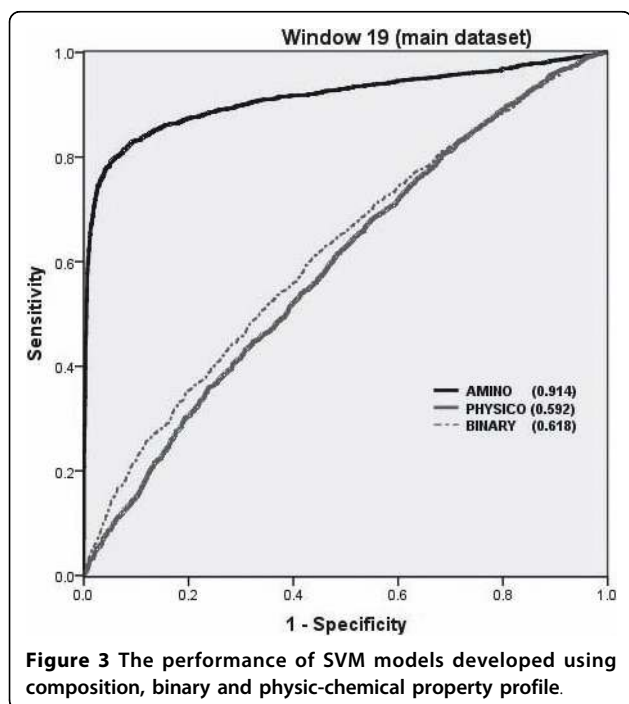
on BPP and achieved MCC 0.06 and 0.44 for BPP and CPP respectively. MCC decreases when we used realistic set of patterns instead of balance set of patterns but accuracy was nearly the same in both cases. In order to compare performance of our model with existing methods we also measured performance in term of AUC. Figure 4 shows the ROC plot of our models on benchmark dataset, we achieved AUC 0.56, 0.57 0.89 for models based on BPP, PPP and CPP respectively. These results demonstrate that CPP based models are more accurate than other models. AUC was more than 0.85 for both set of patterns, realistic and balance (Figure 4). We compared performance of our model with existing methods (Table 5) and observed that our model is as good as any other method. This means our model may complement existing methods and can be used when structure of the antigen is not available.

### Implementation

A user-friendly web server 'CBTOPE' was developed for the prediction of antibody interacting residues or B-cell conformational epitopes. The server is developed using CGI-Perl script, HTML and installed on a Sun Server (420E) under UNIX (Solaris 7) environment. The user may submit the amino acid sequence(s) in 'FASTA' format. The server generates the 19 window patterns of all submitted sequences, calculates amino acid composition and predicts antibody interacting residues. The output is the amino acid sequence mapped with a probability scale ranging from 0 to 9 for each amino acid. 0 indicates the rarest chance of being that residue in a B-cell epitope and 9 as the most probable. We suggest that for high specificity (high confidence) prediction, user should select the higher threshold value but compromising the sensitivity of prediction. However, for maximum prediction of antibody interacting residues user should opt lower threshold. There is always interplay between sensitivity and specificity. The default threshold was set at -0.3 as at this value, sensitivity and specificity was found equal during the development. Web-server is freely available at <http://www.imtech.res.in/raghava/cbtope>.

### Discussion

It has been a great challenge for the academicians to devise algorithms and methods for the identification and mapping of potential B-cell epitopes from an antigen sequence. Much effort has been put in trying to predict the conformational B-cell epitope. Previous methods predict conformational B-cell epitopes with reasonably high accuracy, the limitation of these methods is that they require tertiary structure of the antigen. Experimental technique like X-ray crystallography used for determining structure of a protein is costly, tedious and time consuming. To the best of author's knowledge



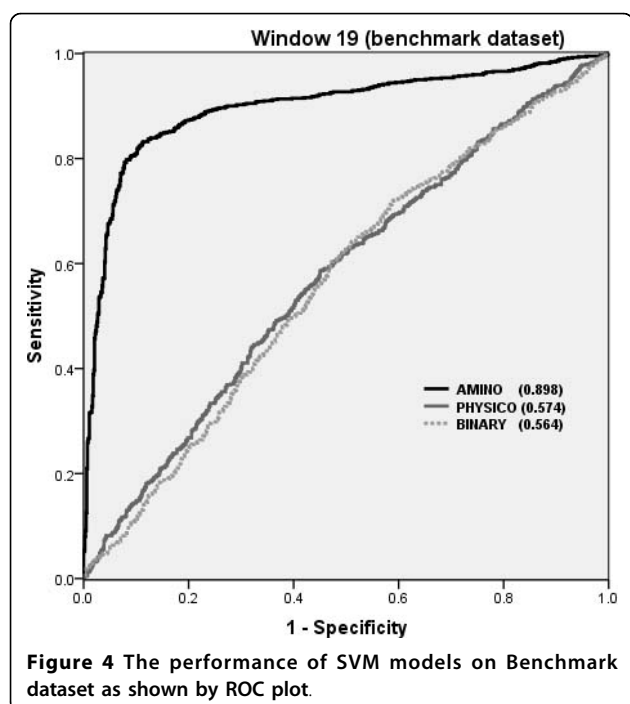
**Table 4 The performance of BPP and CPP based SVM model on Benchmark dataset, developed using balance and realistic set of patterns**

Type of Pattern set	Model	SVM parameters	Thr*	Sen	Spe	Acc	MCC
Realistic	BPP	t 2 g 0.001 j 10 c 10	-0.2	50.49	60.28	59.49	0.06
	CPP	t 2 g 0.001 j 10 c 10	-0.3	80.41	84.64	84.30	0.44
Balance	BPP	t 2 g 0.01 j 1 c 10	0.1	61.31	51.22	56.27	0.13
	CPP	t 2 g 0.01 j 1 c 10	0	82.36	89.42	85.89	0.72

Models were developed using window size 19.

there is no method which can predict conformational B-cell epitopes in an antigen in absence of tertiary structure. There is a need to develop methods for predicting conformational B-cell epitopes in an antigen from its primary sequence. This study describes the method CBTOPE developed for predicting conformational epitopes of antibody interacting residues in antigens. In order to compare performance of our models we chose a benchmark dataset, which was used to evaluate the performance of structure based methods. In order to increase the data we included data from IEDB database. We presumed that the antibody interacting residues are the conformational B-cell epitope residues. We used traditional features of binary and physico-chemical profiles of patterns, evaluated by 5-fold cross validation while using SVM as a classifier. Performance was very poor in BPP models due to the fact that for 21xW vector size only W values represent 1, the rest all are 0 so the noise is more in BPP model. PPP model also could not perform well although it was earlier used for linear and structure based conformational B-cell epitope

prediction. From the preliminary analysis of the composition and 2 sample logo plots of positive and negative patterns, it was clear that there is significant difference in the composition and surface propensities of certain residues which can be exploited to discriminate the patterns. Finally we used for the first time, in our study simple amino acid composition model of patterns (CPP) with vector size of 20 which was evaluated on two different datasets. The performance improved significantly and it is interesting to note that it can be used for the prediction of conformational B-cell epitopes despite the fact that in CPP model we lost the amino acid order information unlike BPP. This problem may be equated to the sub-cellular localization of proteins wherein it was observed that simple amino acid composition model perform better than other features. But unlike sub-cellular localization we exploited composition of patterns instead of whole protein sequence. It should be noted that despite the prediction of antibody interacting or individual B-cell epitope residues, being a sequence based method and the lack of 3D structural input, CBTOPE cannot assist in determining the number and distance needed to make an epitope segment in the antigen sequence. This information can be obtained by mapping of the predicted residues on the modeled structure. We hope that the present model is unique in its kind and will compliment the available structure based methods used for the prediction of antibody interacting residues or conformational B-cell epitopes.



**Figure 4 The performance of SVM models on Benchmark dataset as shown by ROC plot.**

## Conclusion

We showed that simple antigen sequence can be used for the prediction of conformational B-cell epitopes and no structure or homology is required. We introduced for the first time concept of local amino acid composition of antigen. We showed that our CPP composition based SVM model outperformed other structure methods with better sensitivity and AUC on the same benchmark dataset.

## Additional material

**Additional file 1: Additional file for CBTOPE.** Additional file 1 containing BPP and PPP matrix and detailed threshold-wise results of selected windows and kernels.

**Table 5 Overall performance of structure based and CBTOPE algorithms on benchmark dataset**

Evaluation parameter	ProMate	PSI-PRED best patch	Patch Dock best model	ClusPro (DOT) best model	CEP	DiscoTope (-7.7)	CBTOPE* (This Study)
Sen*	0.09	0.33	0.43	0.45	0.31	0.42	<b>0.80</b>
1-Spe	0.08	0.14	0.11	0.07	0.22	0.21	0.15
PPV	0.10	0.19	0.26	0.39	0.11	0.16	0.31
Acc	0.84	0.82	0.85	0.89	0.74	0.75	0.84
AUC	0.51	0.60	0.66	0.69	0.54	0.60	<b>0.89</b>

\* (Thr- Threshold, Sen - Sensitivity, Spe - Specificity, Acc - Accuracy, PPV - positive predictive value)

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#### Authors' contributions

HRA carried out the data analysis and interpretation, developed computer programs, wrote the manuscript and developed the web-server. GPSR conceived and coordinated the project, guided its conception and design, helped in the interpretation of data, refined the drafted manuscript and gave overall supervision to the project. Both authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

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