Application of RotaSVM for HLA Class II Protein-Peptide Interaction Prediction

Shib Sankar Bhowmick[1,2,*], Indrajit Saha[1,*], Giovanni Mazzocco[3], Ujjwal Maulik[1], Luis Rato[2], Debotosh Bhattacharjee[1] and Dariusz Plewczynski[3]

[1]*Department of Computer Science and Engineering, Jadavpur University, Kolkata-700032, West Bengal, India*

[2]*Department of Informatics, University of Evora, 7004-516 Evora, Portugal*

[3]*Interdisciplinary Centre for Mathematical and Computational Modelling, University of Warsaw, 02-106 Warsaw, Poland*

Keywords: HLA Class II, Machine Learning, MHC, Peptide Binding, T Cell Epitopes.

Abstract: In this article, the recently developed RotaSVM is used for accurate prediction of binding peptides to Human Leukocyte Antigens class II (HLA class II) proteins. The HLA II - peptide complexes are generated in the antigen presenting cells (APC) and transported to the cell membrane to elicit an immune response via T-cell activation. The understanding of HLA class II protein-peptide binding interaction facilitates the design of peptide-based vaccine, where the high rate of polymorphisms in HLA class II molecules poses a big challenge. To determine the binding activity of 636 non-redundant peptides, a set of 27 HLA class II proteins are considered in the present study. The prediction of HLA class II - peptide binding is carried out by an ensemble classifier called RotaSVM. In RotaSVM, the feature selection scheme generates bootstrap samples that are further used to create a diverse set of features using Principal Component Analysis. Thereafter, Support Vector Machines are trained with these bootstrap samples with the integration of their original feature values. The effectiveness of the RotaSVM for HLA class II protein-peptide binding prediction is demonstrated in comparison with other traditional classifiers by evaluating several validity measures with the visual plot of ROC curves. Finally, Friedman test is conducted to judge the statistical significance of RotaSVM in prediction of peptides binding to HLA class II proteins.

1 INTRODUCTION

Major Histocompatibility Complex (MHC) molecules play a key role in the activation of the adaptive immune response. They bind and expose an antigen (immunogenic peptide) to T-cell receptors (TCR) triggering an immune response against the infected cell or foreign agent. Human MHC proteins, also known as Human Leukocyte Antigens (HLA), make multiple contacts with the side-chains of binding peptides, defining the binding motif and determine the specificity of binding. There are two classes of HLA molecules: class I and class II. The binding domain of the class I molecules is composed of a single heavy chain, constituting a closed binding groove that accepts only peptides with fixed length of 9 amino acids (AAs). In contrast, class II molecules are composed of two variable chains, with an open binding groove that allows peptides of different length (between 11 and 22 AAs) to bind using different binding frames (Stern and Wiley, 1994). This variability, along with the high degree of polymorphism in HLA class II molecules constitute a challenge for T cell epitope discovery. Even though many of the alleles could be functionally highly related, the binding pockets are alike among different alleles. Generally, it is very difficult to identify such similarities, since subtle differences in binding pocket amino acids (AAs) can lead to dramatic changes in the binding specificity (Nielsen et al., 2007; Saha et al., 2013).

During the last decade, the high level of accuracy in prediction of T cell epitopes makes prediction algorithms a natural and integral part of most major large-scale epitope discovery projects (Sette and Peters, 2007; Lauemoller et al., 2000; Moutaftsi

*These two authors are joint first author and contributed equally.

Bhowmick S., Saha I., Mazzocco G., Maulik U., Rato L., Bhattacharjee D. and Plewczynski D..
Application of RotaSVM for HLA Class II Protein-Peptide Interaction Prediction.
DOI: 10.5220/0004804801780185
In *Proceedings of the International Conference on Bioinformatics Models, Methods and Algorithms* (BIOINFORMATICS-2014), pages 178-185
ISBN: 978-989-758-012-3

et al., 2006). The single most selective event defining T cell epitopes is the binding of peptide fragments to the HLA complexes (Yewdell and Bennink, 1999; Haque and Blum, 2005). Most HLA class II binding prediction methods have been trained and evaluated on very limited data sets covering only a single or a few different HLA class II alleles (Karpenko et al., 2005; Murugan and Dai, 2005; Chang et al., 2006; Salomon and Flower, 2006; Bui et al., 2005; Nielsen et al., 2004; Wan et al., 2006; Brusic et al., 1998). To the best of our knowledge, methods like Propred (Singh and Raghava, 2001) and TEPITOPE (Sturniolo et al., 1999), are experimentally derived virtual matrix-based prediction methods that cover different HLA-DR alleles. NetMHCII (Sturniolo et al., 1999) and ARB (Bui et al., 2005) are weighted matrix data-driven methods that use peptide/MHC binding data of 14 HLA-DR alleles as well as some mouse MHC class II alleles. Very limited work has been done on deriving HLA class II-peptide binding prediction algorithms with broad allelic coverage. Although the development of such methods based on binding information or physiochemical properties of AAs, would represent a significant help in the study of human immune system.

In this article, the above fact motivated us to use recently developed RotaSVM (Bhowmick et al., 2013) for accurate prediction of peptide binding to class II HLA proteins such as DP, DQ and DR. RotaSVM is an ensemble classifier that combines a rotational feature selection scheme with Support Vector Machines (SVMs), in order to produce a predefinite number of SVMs outputs. For each SVM, the training data are generated from the bootstrap samples by splitting the feature set randomly into ξ number of subsets. Subsequently, principal component analysis (PCA) is used for each subset to create new feature sets and all the principal components are retained to preserve the variability information about the training data. Thereafter, such features are used to train a SVM. During the testing phase of RotaSVM, the sample data are the input to the rotation specific SVM. Subsequently, it is classified by computing average posterior probability. The classification is performed on the binding dataset of 27 HLA class II proteins. The performance of the RotaSVM is demonstrated by comparison with the individual Support Vector Machine(SVM) (Vapnik, 1995), Random Forest (RF) (Breiman, 2001), Naive Bayes (NB) (George and Langley, 1995) and K-Nearest Neighbor (K-NN) (Cover and Hart, 1967) classifiers in terms of average accuracy, precision or Positive Predictive value (PPV), (Ramana and Gupta, 2010), recall, F-measure, Matthews correlation coeffcient (MCC) (Ramana and Gupta, 2010), and area under the ROC curve (AUC) values of the random subsample dataset. Goodness of the RotaSVM is judged by computing gain values along with the statistical significance test, called Friedman test (Friedman, 1937; Friedman, 1940).

2 MATERIALS AND METHODS

2.1 Dataset

An enhanced Greenbaum dataset consisting of 27 HLA class II proteins binding 636 peptides obtained from Phleumpratense (Greenbaum et al., 2011) is used in this present work. The dataset consists of IC50 HLA II-peptide binding affinity values. The raw dataset is transformed into binary binding matrices which contain 1 and 0 for binding and non-binding events, respectively. In this regard, for the enhanced Greenbaum dataset, a compatible criterion is adopted, where for considering a peptide as a binder to the HLA class II proteins the maximum IC50 values are chosen around 1000 nM by setting the threshold value for HLA class II at 1000 nM. This stringent IC50 threshold value is adopted in order to decrease the background noise of the data (Greenbaum et al., 2011).

Before going to predict the HLA class II binding peptides, the homogenization of the peptide length is a mandatory step for RotaSVM predictor and therefore in the chosen dataset, homogenized length of 15 AAs for all the peptides is considered. In that homogenized dataset the bordering AAs that exceed 15 AAs peptides are removed. The dissection is selected after an accurate comparative analysis of the less conserved residues within longer peptides (Saha et al., 2013). Thereafter, 40 high-quality AA indices (HQI 40) (Saha et al., 2011a; Plewczynski et al., 2012) are used to encode each peptide, where AA index represents various physicochemical and biochemical properties of AAs in terms of numerical values. Therefore, each peptide is expressed by 15 AAs $\times$ 40 HQI = 600 features.

For this experiment, *Percentage of Positive Activity* (PPA) is computed from binary binding affinity matrix to define the total number of binding events among peptides and each HLA-II DP, DQ and DR protein. To prepare data for this process, initially the highest number of positive activity is computed among all instances and then for each instance the positive activity is computed with respect to this highest PPA. This process is carried out individually for HLA-II DP, DQ and DR proteins. Thereafter, a

threshold label is set and if the PPA of any instance is greater than this threshold value, then the activity value is considered as 1, otherwise it is equal to 0. Each of these activity values is working as an indicator of the peptide binding event. If the activity value is 1, then it is binding to the respective HLA, otherwise not. Since the activity value to an instance is defined with respect to the threshold value, hence a lower threshold gives a higher number of binding peptides. Different incremental threshold values are applied and the statistics are given in Table 1. Since, it is observed that the number of positive and negative binders, play a crucial role for supervised classifiers, hence for RotaSVM, the threshold level at 15%, 30% and 30% are considered to have balanced numbers of positive and negative binders for HLA-II DP, DQ and DR, respectively. The data generation and the experimental procedure are shown through block diagram in Figure 1.

Table 1: Statistics of dataset used in RotaSVM.

HLA II	Threshold Levels (%)	Number of Positives	Number of Negatives	Percentage of Positives (%)
DP	10	325	311	51.10
	15	**325**	**311**	**51.10**
	20	217	419	34.11
	25	217	419	34.11
	30	217	419	34.11
	40	153	483	24.05
	50	108	528	16.98
DQ	10	526	110	82.70
	15	526	110	82.70
	20	330	306	51.88
	25	330	306	51.88
	30	**330**	**306**	**51.88**
	40	158	478	24.84
	50	74	562	11.63
DR	10	518	118	81.44
	15	464	172	72.95
	20	413	223	64.93
	25	413	223	64.93
	30	**356**	**280**	**55.97**
	40	272	364	42.76
	50	218	418	34.27

2.2 RotaSVM for HLA Class II Protein-Peptide Binding Prediction

RotaSVM is a newly developed ensemble classifier, where a set of SVMs is used as base classifier. To construct the new feature set for each SVM, bootstrap samples are extracted from the original training set. Then the feature set is randomly split and linearly transformed to construct new subsets. In addition to this, final feature set is constructed with all the transformed features for each SVM in the ensemble, where the diversity of the RotaSVM is guaranteed by this transformation. Thereafter, the average of posterior probability gives the classification results. Here, as the SVMs are used, the basic idea of SVM is to find a hyperplane which separates the d-dimensional data perfectly into two classes, however, since classification data are often not linearly separable, SVM introduced the notion of a "kernel" which embeds the data into a higher-dimensional feature space where the data are linearly separable.

Consider a training set $\pounds = \{(x_i, y_i)\}_{i=1}^{N}$ consisting of N independent instances, Y be the corresponding label and F be the feature set where each (x_i, y_i) is described by an input attribute vector $x_i = (x_{i1}, x_{i2}, \ldots, x_{id}) \in \mathbb{R}^d$ and a class label y_i. Let X be a $N \times d$ data matrix composed with the values of d input attribute and ω be the set of class labels $\{\omega_1, \omega_2, \ldots, \omega_c\}$, from which Y takes values. Assume that the feature set is split randomly into ξ subsets with approximate size and $\top$ is the ensemble size in the RotaSVM. Here, ξ and $\top$ should be specified in advance.

During the training of each SVM, the feature set F is randomly split into ξ number of disjoint subsets. Subsequently, a submatrix $X_{t,s}$, where t is the timestamp of the SVM classifier runs and s is the subset number, is created with the attributes in $F_{t,s}$. From this submatrix $X_{t,s}$, a bootstrap subset of objects is drawn with the size of 75% of the dataset to form a new training set, which is denoted by $X'_{t,s}$. Thereafter, PCA technique is applied to each subset to obtain a matrix $M_{t,s}$ where all principal components are retained in order to preserve the variability information in the data. Thus, ξ axis rotations take place to form the new attributes for each SVM classifier. Subsequently, the matrix $M_{t,s}$ is arranged into a block diagonal matrix B_t. To construct the training set for classifier SVM_t the columns of B_t is rearranged according to the original feature sequence, and assuming that the rearranged rotation matrix is denoted by B_t^r. The training set for classifier SVM_t is $[XB_t^r, Y]$. Details of RotaSVM are mentioned in Figure 2.

In the testing phase, given a test sample $\varkappa$, let $SVM_{t,i}(\varkappa B_t^r)$ be the posterior probability produced by the classifier SVM_t on the hypothesis that $\varkappa$ belongs to class ω_i. Then the confidence for a class is calculated by the average posterior probability of combined SVMs as follows:

$$\varphi_i(\varkappa) = \frac{1}{\top} \sum_{t=1}^{\top} SVM_{t,i}(\varkappa B_t^r), \quad where\ i = 1, 2, \ldots, c \qquad (1)$$

Thereafter, $\varkappa$ is assigned to the class with the largest confidence.

The RotaSVM is applied to predict the HLA class II-peptide binding, separately for DP, DQ and DR. Random sub-sampling validation is used for this experiment to prepare the training and testing data. According to the used method, the dataset is randomly split into training and testing (validation) data. For each of the selected threshold values of DP, DQ and

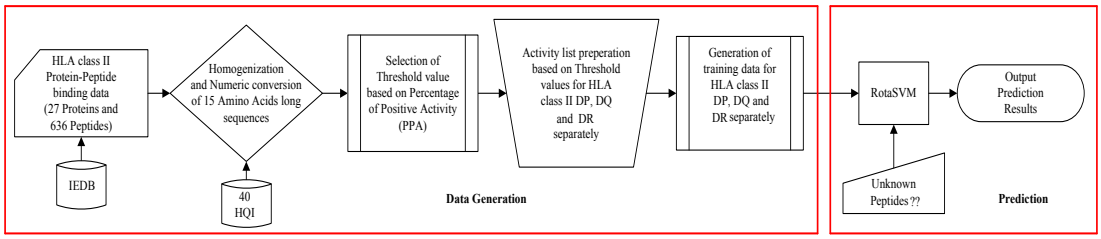


Figure 1: A block diagram of the workflow.

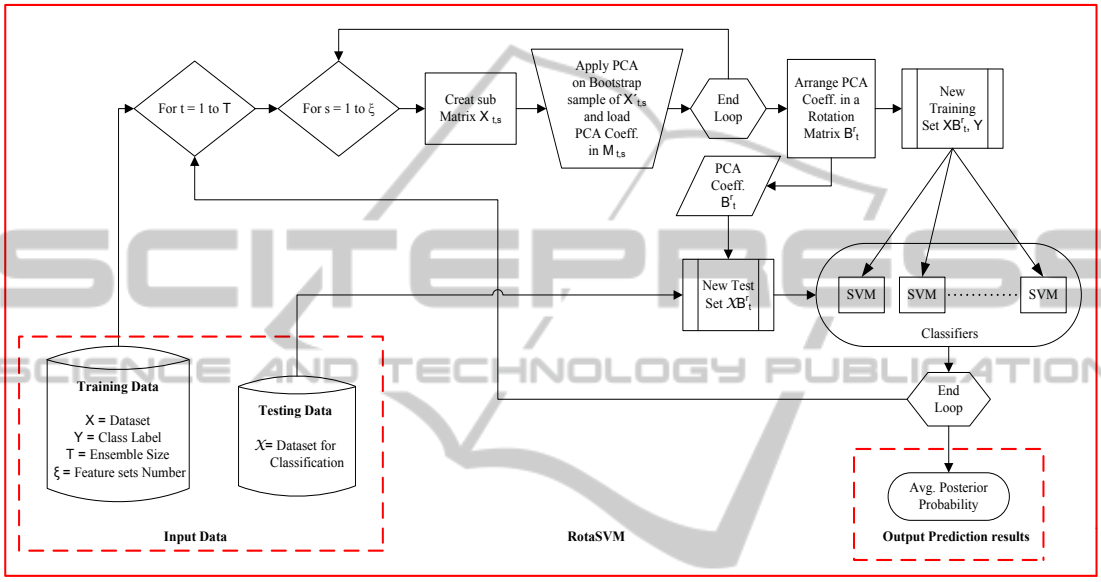


Figure 2: A block diagram of the RotaSVM.

DR, the dataset is randomly split three times. This is done to eliminate the possible bias during the training procedure in any given train/test dataset combination use space. Afterward, the train and test datasets are normalized, where each input data is normalized to the range [0,1]. Thereafter, for each such split, RotaSVM learns the normalized training data and predictive accuracy is assessed using three randomly chosen normalized test data. The results are then averaged over different such splits. Here, two thirds of the dataset is used for training the classifier and rest of the dataset is used for testing. Among 636 peptides, which are responsible for binding to HLA-DP, -DQ and -DR proteins, are separately identified by RotaSVM.

3 RESULTS AND DISCUSSIONS

3.1 Performance Metrics

The performance evaluation of the RotaSVM for HLA class II protein-peptide interaction prediction is here reported. Different measures are used as performance metric for the RotaSVM. These measures can be derived from the following four scalar quantities; TP (true positives: number of correctly predicted peptides that bind HLA class II proteins), TN (true negatives: number of correctly predicted peptide as non-binders of HLA class II proteins), FP (false positives: number of incorrectly predicted peptides that bind HLA class II proteins), FN (false negatives: number of non-correctly predicted peptides as non-binders of HLA class II proteins). The above four measures including the accuracy, precision or PPV, recall or sensitivity, F-measure, MCC, and area under the ROC curve (AUC) values are calculated as follows.

$$Accuracy = \frac{(TP+TN)}{(TP+TN+FP+FN)} \times 100 \quad (2)$$

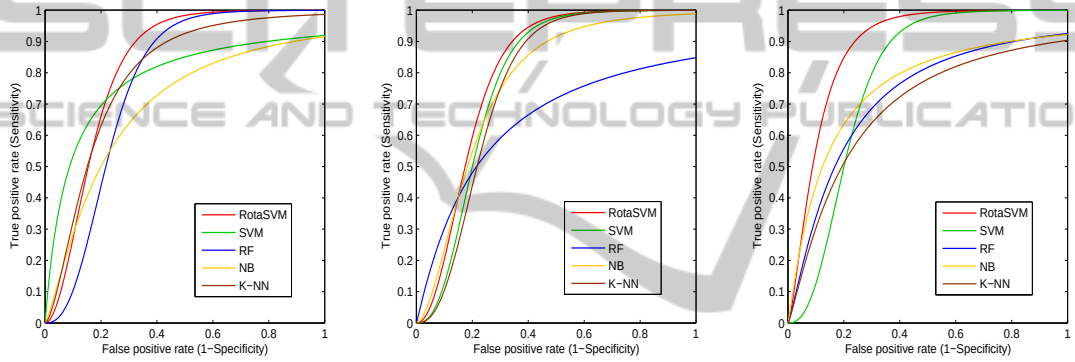
$$Precision = PPV = \frac{TP}{(TP+FP)} \times 100 \quad (3)$$

$$Recall = Sensitivity = \frac{TP}{(TP+FN)} \times 100 \quad (4)$$

$$F\text{-}measure = \frac{(Precision \times Sensitivity)}{(Precision + Sensitivity)} \times 2 \quad (5)$$

Table 2: Performance comparison of RotaSVM based HLA II-peptide binding predictor with other classifiers in terms of average Accuracy, Precision, Recall, F-measure, MCC, PPV and AUC.

Algorithm	HLA II	Accuracy (%)	Precision or PPV	Recall or Sensitivity	F-measure	MCC	AUC
RotaSVM	DP	89.03	91.12	97.09	94.01	0.74	0.82
	DQ	82.44	76.65	99.49	86.59	0.69	0.81
	DR	80.74	80.92	99.07	89.08	0.43	0.88
SVM	DP	80.00	88.17	89.45	88.81	0.40	0.78
	DQ	76.06	76.36	99.49	86.40	0.33	0.78
	DR	78.89	79.18	99.53	88.20	0.37	0.78
RF	DP	77.42	88.39	85.82	87.08	0.36	0.76
	DQ	67.18	77.83	79.80	78.80	0.21	0.64
	DR	75.93	82.82	87.85	85.26	0.34	0.72
NB	DP	74.52	95.79	74.55	83.84	0.34	0.69
	DQ	77.22	77.69	98.48	86.86	0.36	0.77
	DR	76.30	87.50	81.78	84.54	0.35	0.75
K-NN	DP	80.97	89.13	89.45	89.29	0.45	0.79
	DQ	71.04	75.95	90.91	82.76	0.32	0.76
	DR	73.33	80.87	86.92	83.78	0.33	0.69



(i) (ii) (iii)

Figure 3: ROC plots of HLA class II protein-peptide binding prediction for (i) DP, (ii) DQ and (iii) DR.

$$MCC = \frac{(TP \times TN) - (FN \times FP)}{\sqrt{(TP+FN) \times (TN+FP) \times (TP+FP) \times (TN+FN)}} \quad (6)$$

The effectiveness of the RotaSVM results is also justified in terms of gain values. The gain achieved by RotaSVM over other used classifiers is measured. The calculated gain is expressed in percentage:

$$Gain = \frac{(PA\ of\ RotaSVM\ -\ PA\ of\ RC)}{(PA\ of\ RC)} \times 100 \quad (7)$$

where PA signifies the Prediction Accuracy, and RC refers to the Reference Classifier.

In this experiment, the values of ξ and $\top$ for RotaSVM are set to be 3 and 10, respectively as well as the parameters of SVM such as kernel function and the soft margin (cost parameter) are set to be 0.5 and 2.0, respectively. Note that, RBF (Radial Basis Function) kernel is used here for SVM. The K value for the K-NN classifier is chosen as 13 for the satisfactory operation of the classifier. However, to reduce the computational time, we adopted the number of iteration of the RotaSVM to be as 20. The RotaSVM is implemented in Matlab version 2012b.

3.2 Perfomance Analysis of RotaSVM

The overall performance of RotaSVM is computed using three different test sets as described in previous section. Here, 636 peptides are binded to HLA-DP, -DQ and -DR, separately. For HLA-DP the average obtained values of accuracy, precision, recall, F-measure, MCC and AUC are 89.03%, 91.12, 97.09, 94.01, 0.74 and 0.82 respectively, as reported in Table 2. Among three different HLA class II proteins these are the best results produced by RotaSVM in comparison with SVM, RF, NB and K-NN classifiers. RotaSVM provides highest values of accuracy, F-measure and MCC on HLA-DP. In addition, RotaSVM shows equal best values of recall or sensitivity, 99.49, along with the SVM classifier on HLA-DQ. However, it is observed that lower threshold values generate overfitting by producing similar precision and recall values. Hence, 15%, 30% and 30% threshold levels are chosen for DP, DQ and DR respectively, to have balanced number of positive and

negative binding peptides.

ROC curves (Swets, 1988) are plotted here as one of the robust approaches for classifier evaluation. The ROC curves show the trade-off between average true positive rate (sensitivity) and false positive rate (1-specificity) over their entire range of possible values. Furthermore, the performances of each classifier are also measured by AUC, which reflects the ability of the classifiers to discriminate binders from non-binders. We have plotted the ROC curves for all classifiers based on HLA class II protein-peptide binding prediction in Figure 3. RotaSVM has produced best values of AUC for DP, DQ and DR, 0.82, 0.81 and 0.88, respectively in comparison with other methods. These results further reinforced the efficacy of the RotaSVM.

The gain values of RotaSVM over other classifiers are reported in Table 3. Based on obtained accuracy the gains are computed for three different types of HLA class II proteins-peptides binding prediction. The results suggest a positive gain of RotaSVM over all other used classifiers. It demonstrates the effectiveness of RotaSVM in finding HLA class II protein-peptide binding interaction.

Table 3: Gain values of RotaSVM in comparison with other classifiers.

HLA II	SVM (%)	RF (%)	NB (%)	K-NN (%)
DP	11.29	15.00	19.48	09.96
DQ	08.39	22.71	06.76	16.04
DR	02.35	06.34	05.83	10.10

3.3 Statistical Analysis

Statistical significance of RotaSVM results with respect to other classifiers is analysed by using the Friedman test (Friedman, 1937; Friedman, 1940), at the 5% significance level. Friedman test is a non-parametric test, where accuracy values of 20 runs for three difference HLA-DP, -DQ and -DR are considered. According to Friedman test it is assumed that, for a null hypothesis there is no significant difference between the accuracy values of different groups. Whereas, according to the alternative hypothesis it is considered that there is a strong significant difference in the accuracy values within the groups. Table 4 reports the rank of each individual classifier for HLA-DP, -DQ and -DR as well as the average rank of each classifier. Moreover, the results in Table 5 reveal average Chi-Square value and corresponding *p*-value are 47.713 and 0.142×10^{-5}, respectively, which indicate the acceptance of alternative hypothesis. That means, the average accuracy values produced by RotaSVM are statistically significant for all proteins, and this fact remarks the significant superiority of the RotaSVM for predicting HLA class II protein-peptide binding activity.

Table 4: The Friedman ranks of all classifiers.

HLA II	RotaSVM	SVM	RF	NB	K-NN
DP	2.00	3.47	3.53	4.00	3.47
DQ	2.43	3.93	5.00	3.29	4.42
DR	2.92	3.65	3.77	3.99	4.14
Average Rank	2.45	3.68	4.10	3.76	4.01

Table 5: The results of Friedman test.

HLA II	Chi-Square value	*p*-value
DP	71.802	0.111×10^{-5}
DQ	32.754	0.100×10^{-5}
DR	38.584	0.215×10^{-5}
Average	47.713	0.142×10^{-5}

4 CONCLUSIONS

This article demonstrates the effectiveness of the ensemble classier, called RotaSVM, with the use of principal component analysis to create new feature sets for prediction of binding peptides to Human Leukocyte Antigens class II proteins. The effectiveness of the RotaSVM is shown by comparing it with the traditional machine learning algorithms like support vector machine, random forest, naive bayes and K-nearest neighbor in terms of average precision, recall, accuracy, F-measure, Matthew's correlation coefficient and area under the ROC curve values. Finally, the goodness of the RotaSVM for predicting HLA class II protein-peptide binding activity is also shown by computing the gain values along with the statistical significance test. From the results, it can be concluded that the RotaSVM achieved maximum 22.71% gain over Random Forest classifier for HLA-DQ and average gain of 11.19% over all the classifiers for three HLA class II proteins.

For future scope of research, this RotaSVM can be applied to facilitate the laboratory experimental work, when there is the need of HLA specific protein-peptide binding prediction. In addition to this, feature selection and classification play a crucial role in Microarray data analysis (Saha et al., 2011b; Saha et al., 2012; Saha et al., 2011d), pixel classification of satellite images (Saha et al., 2011c; Maulik and Saha, 2010) and other fields of engineering and science (Maulik et al., 2010; Saha et al., 2010; Sur et al., 2009; Saha and Mukhopadhyay, 2008). In such cases, application of RotaSVM would be interesting

to study. Currently, the authors are working in this direction.

ACKNOWLEDGEMENTS

This work is partially supported by Erasmus Mundus Mobility with Asia (EMMA) grant 2012 from the European Union at the Department of Informatics, University of Evora in Portugal and University with Potential for Excellence (UPE)-Phase II project grant from University Grants Commission (UGC) in India.

REFERENCES

Bhowmick, S. S., Saha, I., Rato, L., and Bhattacharjee, D. (2013). RotaSVM: A new ensemble classifier. *Advances in Intelligent Systems and Computing*, 227:47–57.

Breiman, L. (2001). Random forests. *Machine Learning*, 45(1):5–32.

Brusic, V., Rudy, G., Honeyman, G., Hammer, J., and L, L. H. (1998). Prediction of MHC class II-binding peptides using an evolutionary algorithm and artificial neural network. *Bioinformatics*, 14:121–130.

Bui, H. H., Sidney, J., Peters, B., Sathiamurthy, M., Sinichi, A., Purton, K. A., Moth, B. R., Chisari, F. V., Watkins, D. I., and Sette, A. (2005). Automated generation and evaluation of specific MHC binding predictive tools: ARB matrix applications. *Immunogenetics*, 57:304–314.

Chang, S. T., Ghosh, D., Kirschner, D. E., and Linderman, J. J. (2006). Peptide length-based prediction of peptide-MHC class II binding. *Bioinformatics*, 22:2761–2767.

Cover, T. and Hart, P. (1967). Nearest neighbor pattern classification. *IEEE Transactions on Information Theory*, 13(1):21–27.

Friedman, M. (1937). The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *Journal of the American Statistical Association*, 32:675–701.

Friedman, M. (1940). A comparison of alternative tests of significance for the problem of m rankings. *Annals of Mathematical Statistics*, 11:86–92.

George, H. and Langley, J. P. (1995). Estimating continuous distributions in bayesian classifiers. *in Proceedings of the Eleventh Conference on Uncertainty in Artificial Intelligence*, 69:338–345.

Greenbaum, J., Sidney, J., Chung, J., Brander, C., Peters, B., and Sette, A. (2011). Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes. *Immunogenetics*, 63(6):325–335.

Haque, A. and Blum, J. S. (2005). New insights in antigen processing and epitope selection: development of novel immunotherapeutic strategies for cancer, autoimmunity and infectious diseases. *Journal of Biological Regulators and Homeostatic Agents*, 19:93–104.

Karpenko, O., Shi, J., and Dai, Y. (2005). Prediction of MHC class II binders using the ant colony search strategy. *Artificial Intelligence in Medicine*, 35:147–156.

Lauemoller, S. L., Kesmir, C., Corbet, S. L., Fomsgaard, A., Holm, A., Claesson, M. H., Brunak, S., and Buus, S. (2000). Identifying cytotoxic T cell epitopes from genomic and proteomic information. *Rev Immunogenet*, 2:447–491.

Maulik, U., Bandyopadhyay, S., and Saha, I. (2010). Integrating clustering and supervised learning for categorical data analysis. *IEEE Transactions on Systems, Man and Cybernetics Part-A*, 40(4):664–675.

Maulik, U. and Saha, I. (2010). Automatic fuzzy clustering using modified differential evolution for image classification. *IEEE Transactions on Geoscience and Remote Sensing*, 48(9):3503–3510.

Moutaftsi, M., Peters, B., Pasquetto, V., Tscharke, D. C., Sidney, J., Bui, H. H., Grey, H., and Sette, A. (2006). A consensus epitope prediction approach identifies the breadth of murine T(CD8+)-cell responses to vaccinia virus. *Nature Biotechnology*, 24:817–819.

Murugan, N. and Dai, Y. (2005). Prediction of MHC class II binding peptides based on an iterative learning model. *Immunome Research*, 1:6.

Nielsen, M., Lundegaard, C., Blicher, T., Lamberth, K., Harndahl, M., and et al. (2007). NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence. *PLoS ONE*, 2:e796.

Nielsen, M., Lundegaard, C., Worning, P., Hvid, C. S., Lamberth, K., Buus, S., Brunak, S., and Lund, O. (2004). Improved prediction of MHC class I and class II epitopes using a novel gibbs sampling approach. *Bioinformatics*, 20:1388–1397.

Plewczynski, D., Basu, S., and Saha, I. (2012). AMS 4.0: consensus prediction of post-translational modifications in protein sequences. *Amino Acid*, 43(2):573–582.

Ramana, J. and Gupta, D. (2010). Machine learning methods for prediction of CDK-Inhibitors. *PLoS ONE*, 5(10).

Saha, I., Maulik, U., Bandyopadhyay, S., and Plewczynski, D. (2011a). Fuzzy clustering of physicochemical and biochemical properties of amino acids. *Amino Acid*, 43(2):583–594.

Saha, I., Maulik, U., Bandyopadhyay, S., and Plewczynski, D. (2011b). Improvement of new automatic differential fuzzy clustering using SVM classifier for microarray analysis. *Expert Systems with Applications*, 38(12):15122–15133.

Saha, I., Maulik, U., Bandyopadhyay, S., and Plewczynski, D. (2011c). SVMeFC: SVM ensemble fuzzy clustering for satellite image segmentation. *IEEE Geoscience and Remote Sensing Letters*, 9(1):52–55.

Saha, I., Maulik, U., Bandyopadhyay, S., and Plewczynski, D. (2011d). Unsupervised and supervised learning approaches together for microarray analysis. *Fundamenta Informaticae*, 106(1):45–73.

Saha, I., Mazzocco, G., and Plewczynski, D. (2013). Consensus classification of human leukocyte antigen class II proteins. *Immunogenetics*, 65(2):97–105.

Saha, I. and Mukhopadhyay, A. (2008). Improved crisp and fuzzy clustering techniques for categorical data. *IAENG International Journal of Computer Science*, 35(4):438–450.

Saha, I., Plewczynski, D., Maulik, U., and Bandyopadhyay, S. (2010). Real-coded differential crisp clustering for MRI brain image segmentation. *in Proceedings of the IEEE Congress on Evolutionary Computation*, pages 3912–3919.

Saha, I., Plewczynski, D., Maulik, U., and Bandyopadhyay, S. (2012). Improved differential evolution for microarray analysis. *International journal of data mining and bioinformatics*, 6(1):86–103.

Salomon, J. and Flower, D. R. (2006). Predicting class II MHC-peptide binding: a kernel based approach using similarity scores. *BMC Bioinformatics*, 7:501.

Sette, A. and Peters, B. (2007). Immune epitope mapping in the post-genomic era: lessons for vaccine development. *Current Opinion in Immunology*, 19:106–110.

Singh, H. and Raghava, G. P. (2001). Propred: prediction of HLA-DR binding sites. *Bioinformatics*, 17:1236–1237.

Stern, L. J. and Wiley, D. C. (1994). Antigenic peptide binding by class I and class II histocompatibility proteins. *Structure*, 2(4):245–251.

Sturniolo, T., Bono, E., Ding, J., Raddrizzani, L., Tuereci, O., Sahin, U., Braxenthaler, M., Gallazzi, F., Protti, M. P., Sinigaglia, F., and Hammer, J. (1999). Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. *Nature Biotechnology*, 17:555–561.

Sur, A., Patra, N., Chakraborty, S., and Saha, I. (2009). A new wavelet based edge detection technique for iris imagery. *IEEE International Conference on Advance Computing Conference*, pages 120–124.

Swets, J. A. (1988). Measuring the accuracy of diagnostic systems. *Science*, 240:1285–1293.

Vapnik, V. N. (1995). *The Nature of Statistical Learning Theory*. Springer.

Wan, J., Liu, W., Xu, Q., Ren, Y., Flower, D. R., and Li, T. (2006). SVRMHC prediction server for MHC-binding peptides. *BMC Bioinformatics*, 7:463.

Yewdell, J. W. and Bennink, J. R. (1999). Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. *Annual Review of Immunology*, 17:51–88.