



Structural bioinformatics

An ensemble approach to predict binding hotspots in protein–RNA interactions based on SMOTE data balancing and Random Grouping feature selection strategies

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Abstract

Motivation: The identification of binding hotspots in protein–RNA interactions is crucial for understanding their potential recognition mechanisms and drug design. The experimental methods have many limitations, since they are usually time-consuming and labor-intensive. Thus, developing an effective and efficient theoretical method is urgently needed.

Results: Here, we present SREPRHot, a method to predict hotspots, defined as the residues whose mutation to alanine generate a binding free energy change ≥ 2.0 kcal/mol, while others use a cutoff of 1.0 kcal/mol to obtain balanced datasets. To deal with the dataset imbalance, Synthetic Minority Over-sampling Technique (SMOTE) is utilized to generate minority samples to achieve a dataset balance. Additionally, besides conventional features, we use two types of new features, residue interface propensity previously developed by us, and topological features obtained using node-weighted networks, and propose an effective Random Grouping feature selection strategy combined with a two-step method to determine an optimal feature set. Finally, a stacking ensemble classifier is adopted to build our model. The results show SREPRHot achieves a good performance with SEN, MCC and AUC of 0.900, 0.557 and 0.829 on the independent testing dataset. The comparison study indicates SREPRHot shows a promising performance.

Availability and implementation: The source code is available at <https://github.com/ChunhuaLiLab/SREPRHot>.

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Supplementary information: [Supplementary data](#) are available at *Bioinformatics* online.

1 Introduction

Protein–RNA interactions play critical roles in a variety of biological processes by regulating different steps of the gene expression process, from transcription to translation (Keene, 2007). The abnormalities in the interactions may lead to multiple diseases such as cancer and neurological disorders (Lukong *et al.*, 2008). It is known that a small fraction of interfacial residues, termed as binding hotspots, contribute to the majority of binding free energy for target RNA (Clackson and Wells, 1995). Thus, the reliable hotspot identification in protein–RNA interactions is crucial for understanding the potential recognition mechanism and for designing drugs. Experimentally, a hotspot residue can be found by evaluating the binding free energy

change ($\Delta\Delta G$) upon mutating it to alanine (Krüger *et al.*, 2018). However, these methods are costly and time-consuming, and thus developing an effective and efficient computational method is urgently needed to allow for the hotspot identification on a large scale.

Until now, few methods have been developed for predicting hotspots in protein–RNA interactions, which lags behind protein–protein hotspot prediction, because of the limited available experimental data. In 2016, Barik *et al.* proposed HotSPRing, a Random Forest (RF) model which uses structural and physicochemical features of interfacial residues to predict the ranges of $\Delta\Delta G$ for RNA-binding residue mutations. The method gives a Matthews correlation coefficient (MCC) of 0.258 where a cutoff threshold of $\Delta\Delta G = 1.0$ kcal/mol is used. In 2018, Pan *et al.* developed PrabHot,

a better performance tool with MCC being 0.389, which utilizes Boruta (Kursa *et al.*, 2010) feature selection algorithm and a voting machine composed of three different classifiers. Later in 2019, XGBPRH method was introduced by Deng *et al.* (2019), which adopted McTWO algorithm (Ge *et al.*, 2016) to select out six optimal features to train eXtreme Gradient Boosting (XGBoost) classifier (Chen and Guestrin, 2016), and achieves an MCC improvement to 0.661. Despite the advances, the computational prediction of RNA-binding hotspots is still in its infancy.

Besides classifiers, the features used for hotspot prediction are also important. The existing methods mainly use some sequence- and structure-based features. In fact, there are still other features we need to explore to improve the protein–RNA-binding hotspot prediction. In a previous work, we extracted a residue-nucleotide pairwise propensity potential from protein–RNA interactions, which shows a good performance in protein–RNA interaction prediction (Wang *et al.*, 2021), discrimination of near-native complex structures (Li *et al.*, 2012; Lu *et al.*, 2020; Zhang *et al.*, 2017) and identification of interfacial residues (Liu *et al.*, 2021a). The hotspots are a type of special sites at the interface, and therefore we think the propensity potential could be a good feature for identifying hotspots. In addition, as for the residue topological features from amino acid network (AAN) models, they have been successfully used to explore the functional sites, including catalytic, allosteric and ligand binding residues (Yan *et al.*, 2014). Usually, the topological features are obtained from the traditional unweighted AAN model that ignores the residue node heterogeneity which is critical to the discrimination of the structurally or functionally important residues. In view of this, many weighted AAN models have been developed, among which the node-weighted networks developed by Yan *et al.* (2018) are quite able to characterize the node heterogeneity and have been widely applied in the functional residue prediction. Thus, we think that the use of residue topological features from the node-weighted networks will probably have a positive role for hotspot prediction.

Additionally, for a relatively small sample size, selecting a subset of significant features is important for building an effective predictor. The commonly used feature selection methods include minimum Redundancy Maximum Relevance (mRMR; Peng *et al.*, 2005), RF (Breiman, 2001) and Boruta whose performances are not very ideal for a small sample size. Our strategy to solve this problem is that first the samples are divided into several subsets and the feature selection is performed on all the subsets, respectively, and then the commonly selected features are retained as the optimal feature set. Our results demonstrate the effectiveness of the strategy and we call it Random Grouping feature selection strategy in the following.

Another point which needs to be mentioned is the class imbalance problem. To overcome it, most of the existing methods choose $\Delta\Delta G = 1.0$ kcal/mol as the threshold to define the hotspots, and thus the ratio between the numbers of positive and negative samples is close to 1:1. However, Krüger *et al.* (2018) speculate that there are in fact only about 10% hotspots ($\Delta\Delta G \geq 2.0$ kcal/mol) in protein–RNA interfaces. Such a high class imbalance will seriously affect the performance of classifier models, inducing an overfitting to the majority class samples (Chawla *et al.*, 2002). Usually, the over-sampling and under-sampling techniques are used to preprocess the imbalanced data, among which the Synthetic Minority Over-sampling Technique (SMOTE) is often used in the field of commercial data mining (Chawla *et al.*, 2002). Different from the naive random over-sampling algorithms that generate minority class samples through a simple random replication, the SMOTE method generates the synthetic samples via some operations in the feature space, which avoids the overfitting problem to some extent (Chawla *et al.*, 2002). Several recent studies have successfully utilized SMOTE to effectively improve the predictions of protein–protein interaction sites (Wang *et al.*, 2019) and drug–target interactions (Redkar *et al.*, 2020).

In this work, we propose an effective method called SREPRHot (a SMOTE and Random Grouping strategies-based Ensemble learning model for Protein–RNA-binding Hotspot prediction) to predict binding hotspots in protein–RNA interactions, where a threshold of $\Delta\Delta G = 2.0$ kcal/mol is adopted to define a hotspot. The SMOTE

algorithm is introduced to balance the data classes. A subset of optimal features is selected out by our proposed Random Grouping feature selection strategy combined with a two-step method from eight types of candidate features extracted from protein sequences and structures, including the residue-nucleotide pairwise propensity potential and residue topological features from node-weighted AAN models. These features are then utilized to train a stacking ensemble classifier (SCE) to build the hotspot predictor. The framework of SREPRHot method is shown in Figure 1.

2 Materials and methods

2.1 Training and testing datasets

We collected the residue mutation thermodynamics data from two sources: the dbAMEPNI database (database of Alanine Mutagenic Effects for Protein-Nucleic Acids Interaction, <http://zhulab.ahu.edu.cn/dbAMEPNI>; Liu *et al.*, 2018) and the data gathered in developing the hotspot prediction methods HotSPRing, PrabHot and XGBPRH, which were published in 2016, 2018 and 2019, respectively. Thus, we collected 334 residue mutations from 81 complexes in total. To remove the redundancy, the proteins with sequence similarity >40% were excluded by using CD-HIT (Li and Godzik, 2006). After that, only the interfacial residues were retained whose absolute solvent accessibility changes ΔASA [calculated by Naccess (Hubbard and Thornton, 1993)] after binding with target RNAs are $>1.0 \text{ \AA}^2$ (Zhang *et al.*, 2020). Finally, we obtained 229 residue mutations across 58 complexes. Among them, the 15 complexes used as the testing dataset by PrabHot and XGBPRH method are considered as our independent testing dataset for easy comparison with them, and the remaining ones are used as the training dataset (Supplementary Table S1).

Different from the existing methods where the interface residues with $\Delta\Delta G \geq 1.0$ kcal/mol are considered as hotspots, our method adopts the criterion of $\Delta\Delta G \geq 2.0$ kcal/mol. Thus, there are 35 positive and 136 negative samples in the training dataset, and the corresponding numbers are 10 and 48 in the independent testing dataset.

2.2 Feature extraction

A comprehensive set of 120 features from eight types was extracted (Supplementary Table S2). More details on the features are described below.

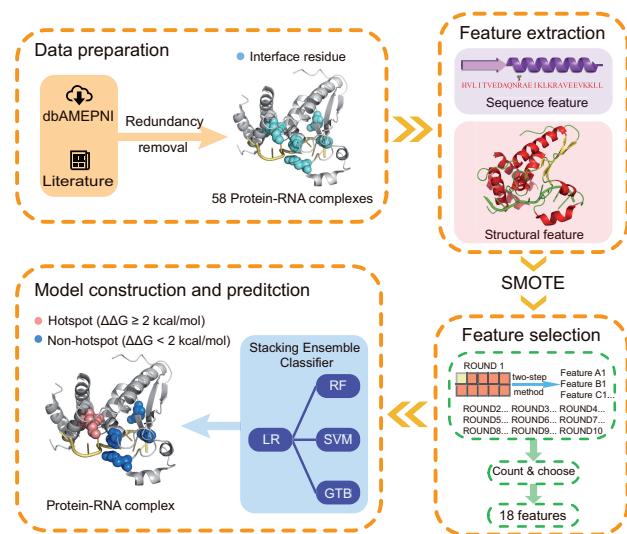


Fig. 1. Framework of SREPRHot for identifying binding hotspots in protein–RNA interactions

2.2.1 Physicochemical characteristics of amino acids

Ten physicochemical properties of amino acids (Supplementary Table S3) are taken from the AAIndex database (Kawashima *et al.*, 2008) and the literatures (Jones and Thornton, 1997a; Li *et al.*, 2008; Ramachandran and Antoniou, 2008; Voet and Voet, 2004), including number of atoms, number of electrostatic charges, number of potential hydrogen bonds, hydrophobicity, hydrophilicity, propensity, isoelectric point, mass, expected number of contacts within a 14 Å sphere and electron-ion interaction potential, which are highly correlated with the interface properties of a protein.

2.2.2 Secondary structural features

SPIDER3 (Heffernan *et al.*, 2015) is applied to compute protein secondary structural features including the main-chain torsional angles (φ and ψ), the main-chain angles between C α atoms (θ and τ) and the probabilities of three kinds of secondary structures: alpha-helix, beta-strand and random coil.

2.2.3 Depth index and protrusion index

The geometric shape complementarity at the binding interface is important for protein–RNA interactions. Depth index (DPX) and protrusion index (CX) were proposed to characterize the embedded and protruding conditions of an atom surrounded by other non-hydrogen atoms, respectively (Pintar *et al.*, 2002, 2003). We use PSAIA (Mihel *et al.*, 2008) to calculate the indexes for a protein in bound and unbound states including the means of DPXs and CXs of all atoms of a residue and their standard deviations, and the means of DPXs and CXs of side-chain atoms and their standard deviations. In addition, the differences in the means and standard deviations of all atoms and side-chain atoms of a residue between bound and unbound states are also computed.

2.2.4 Solvent accessible surface area

The solvent accessible surface areas (SASAs) of a residue in the complex and monomer are calculated by Naccess for a total of 10 attributes: absolute and relative values for all atoms, total side-chain atoms, main-chain atoms, non-polar atoms and all polar atoms in a residue. Moreover, their changes and the corresponding square roots between the two states are also calculated.

2.2.5 Position-specific scoring matrix

The position-specific scoring matrix (PSSM) gives the probability of occurrence of each kind of amino acid residue at each position, which reflects the evolutionary information of a residue position (Liu *et al.*, 2021b). For a protein with N residues, the size of its PSSM matrix is $N \times 20$ and each row encapsulates the evolutionary information for a residue position. The PSSM of a protein is calculated by PSI-BLAST (Altschul *et al.*, 1997) searching against NCBI non-redundant protein sequence database.

2.2.6 Solvent exposure features

Half-sphere exposure (HSE; Hamelryck, 2005), a kind of solvent exposure measures that describes the contacts between residues and solvent molecules, has been proved to be important for protein structure and function predictions (Sharma *et al.*, 2019). HSE is a two-dimensional measure, where a residue's spatial sphere is divided into two half parts: HSE-up (the upper sphere in the direction of the side chain of a residue) and HSE-down (the lower sphere in the opposite direction). HSEpred (Song *et al.*, 2008) is employed to compute the solvent exposure features HSE-up and HSE-down, and in addition the residue contact number (CN) is also calculated.

2.2.7 Residue interface propensity

Residue interface propensity (IP) is from our previously obtained 20×4 residue-nucleotide pairwise propensity potential that was extracted from 251 protein–RNA interactions (Li *et al.*, 2012), and was later updated (used here, Supplementary Table S4) based on a larger dataset including 694 interactions (Lu *et al.*, 2020). The

propensity of one residue-nucleotide pair is obtained from its observed probability divided by its expected probability of occurring on the interfaces. Here, the IP of a residue type is represented as an average of its paired propensities over the four kinds of nucleotides.

2.2.8 Residue topological features from AAN

Compared with the traditional unweighted AAN, the node-weighted AAN, which considers residue heterogeneity, can better reflect the residue topological properties (Yan *et al.*, 2018). Here, besides the unweighted AAN, the four node-weighted AANs based on residue mass, hydrophobicity, polarity and solvent accessibility, respectively, are constructed and the corresponding residue topological features including degree, betweenness centrality and closeness centrality are calculated by using the R package 'NACEN' (Yan *et al.*, 2018).

2.3 SMOTE dataset balancing algorithm

For the data class imbalance problem, the SMOTE is utilized to generate the minority positive samples to achieve the class balance. First the k-nearest neighbors (kNNs) y (here the default $k = 5$ adopted) of a sample x in the minority class are found, and then new samples are built by the random interpolation operation according to the following equation:

$$x_{\text{new}} = x + (y - x) \times \delta, \quad (1)$$

where δ is a random number within the interval of (0, 1).

2.4 Feature selection

Here, we propose a new Random Grouping strategy combined with a two-step algorithm to select the optimal feature subset. First, the training dataset is randomly divided into 10 equal groups and the feature selection is performed 10 rounds with 9 groups of the 10 used for each round. Then, the selected features in each round are recorded and only the features selected not less than 2 times in the 10 rounds are finally retained as the optimal feature set.

For each round, a two-step method is adopted. First mRMR and Decision Tree (DT) methods (Quinlan, 1979) are combined to sort the importance of the candidate features. Then, Sequential Forward Selection (SFS; Kohavi and John, 1997) combined with Support Vector Machine (SVM) with default parameters is used to determine the optimal feature combination from the top 60 in the importance list through maximizing the E_c score (Pan *et al.*, 2020) via 10-fold cross-validation repeated 5 times. The E_c score is calculated as

$$E_c = \frac{1}{R} \sum_{j=1}^R \left[\frac{1}{n} \sum_{i=1}^n (\text{ACC}_{ij} + \text{SEN}_{ij} + \text{SPE}_{ij} + \text{MCC}_{ij} + \text{AUC}_{ij}) \right], \quad (2)$$

where n and R (10 and 5 adopted) are the number of cross-validation folds and the times of the n -fold cross-validation, respectively, and ACC, SEN, SPE, MCC and AUC are the values of accuracy, sensitivity, specificity, Matthew's correlation coefficient and AUC score, respectively. Supplementary Figure S1 shows the flowchart of our feature selection process.

2.5 Stacking ensemble classifier

Stacking (Wolpert, 1992), an ensemble learning strategy that combines multiple base classifiers via a meta-classifier, has been proved to perform better than the single classifiers by many researches. Here, we apply three boost classifiers Gradient Tree Boosting (GTB; Friedman, 2002), RF (Breiman, 2001) and SVM (Cherkassky, 1997), as base classifiers, and Logistic Regression (LR; Wright, 1995) as the meta-classifier.

2.6 Performance evaluation

SREPRHot is tuned on the training dataset by a 10-fold cross-validation, and tested on the independent testing set. The evaluation indicators including accuracy (ACC), sensitivity (SEN), specificity

Table 1. Prediction results from models trained on balanced training datasets by SMOTE and Random Repeat Oversampling techniques, respectively, and on the initial imbalanced one

Data	ACC	SEN	SPE	PRE	F1	MCC	AUC
Imbalanced	0.795 ± 0.092	0.492 ± 0.254	0.874 ± 0.076	0.513 ± 0.264	0.490 ± 0.241	0.371 ± 0.294	0.691 ± 0.137
Balanced by Random Repeat Oversampling	0.825 ± 0.078	0.633 ± 0.176	0.874 ± 0.094	0.642 ± 0.215	0.601 ± 0.123	0.515 ± 0.167	0.780 ± 0.096
Balanced by SMOTE	0.833 ± 0.091	0.800 ± 0.227	0.847 ± 0.113	0.602 ± 0.231	0.646 ± 0.171	0.581 ± 0.208	0.848 ± 0.145

Table 2. Prediction results of the models using classical feature selection methods and Random Grouping strategy combined with a two-step method

Method	ACC	SEN	SPE	PRE	F1	MCC	AUC
mRMR (-)	0.840 ± 0.061	0.625 ± 0.314	0.861 ± 0.102	0.527 ± 0.231	0.554 ± 0.205	0.472 ± 0.201	0.807 ± 0.105
mRMR (+)	0.823 ± 0.089	0.673 ± 0.237	0.853 ± 0.131	0.610 ± 0.225	0.618 ± 0.131	0.523 ± 0.175	0.805 ± 0.136
RF (-)	0.836 ± 0.100	0.580 ± 0.365	0.870 ± 0.110	0.522 ± 0.342	0.534 ± 0.318	0.446 ± 0.340	0.798 ± 0.191
RF (+)	0.857 ± 0.098	0.615 ± 0.242	0.885 ± 0.092	0.584 ± 0.188	0.580 ± 0.180	0.507 ± 0.207	0.830 ± 0.126
Boruta (-)	0.845 ± 0.067	0.683 ± 0.358	0.883 ± 0.084	0.534 ± 0.288	0.586 ± 0.281	0.503 ± 0.296	0.812 ± 0.135
Boruta (+)	0.849 ± 0.099	0.693 ± 0.248	0.889 ± 0.107	0.592 ± 0.237	0.620 ± 0.188	0.541 ± 0.249	0.822 ± 0.132
SFS (-)	0.810 ± 0.086	0.665 ± 0.242	0.837 ± 0.092	0.557 ± 0.188	0.576 ± 0.180	0.482 ± 0.207	0.809 ± 0.128
SFS (+)	0.821 ± 0.081	0.628 ± 0.299	0.868 ± 0.073	0.621 ± 0.198	0.588 ± 0.218	0.501 ± 0.265	0.837 ± 0.204
Two-step (-)	0.831 ± 0.117	0.768 ± 0.292	0.843 ± 0.137	0.522 ± 0.298	0.591 ± 0.279	0.525 ± 0.292	0.848 ± 0.132
Two-step (+)	0.833 ± 0.091	0.800 ± 0.227	0.847 ± 0.113	0.602 ± 0.231	0.646 ± 0.171	0.581 ± 0.208	0.848 ± 0.145

Note: (+) and (-): with and without Random Grouping strategy. mRMR, minimum redundancy maximum relevance; RF, random forest; SFS, sequential forward selection.

(SPE), precision (PRE), F1 score (F1) and MCC are used, which are defined as follows:

$$\text{SEN} = \frac{\text{TP}}{\text{TP} + \text{FN}}, \quad (3)$$

$$\text{SPE} = \frac{\text{TN}}{\text{TN} + \text{FP}}, \quad (4)$$

$$\text{PRE} = \frac{\text{TP}}{\text{TP} + \text{FP}}, \quad (5)$$

$$F_1 = \frac{2 \times \text{SEN} \times \text{PRE}}{\text{SEN} + \text{PRE}}, \quad (6)$$

$$\text{ACC} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{TN} + \text{FP} + \text{FN}}, \quad (7)$$

$$\text{MCC} = \frac{\text{TP} \times \text{TN} - \text{FP} \times \text{FN}}{\sqrt{(\text{TP} + \text{FP})(\text{TP} + \text{FN})(\text{TN} + \text{FP})(\text{TN} + \text{FN})}}, \quad (8)$$

where the true positive (TP), false positive (FP), true negative (TN) and false negative (FN) are obtained by comparing the predicted label for each residue with the actual one. We also use the area under a Receiver Operating Characteristic (ROC) curve, named AUC, to measure the performance of the model.

3 Results

3.1 Advantage of the SMOTE algorithm

The use of the criterion of hotspots $\Delta\Delta G \geq 2.0$ kcal/mol leads to a high imbalance between the positive and negative samples, which makes the feature selection and model construction largely dominated by negative samples, therefore disadvantageous for model construction. The SMOTE algorithm was adopted to generate the minority (positive) class samples in the training set to balance the data. In order to explore whether balancing the positive and negative samples to the ratio 1:1 can improve the model performance,

we compared the results obtained by the models trained on the balanced data by SMOTE and by Random Repeat Oversampling technique (simple copying operations), and on the initial imbalanced ones, as shown in **Table 1**.

From **Table 1**, compared with the result from the model trained on the imbalanced data, the corresponding results on the balanced ones have an evident improvement. Furthermore, the model trained on the dataset processed by SMOTE achieves a better prediction than that processed by Random Repeat Oversampling, with SEN, MCC and AUC improved by 26.4%, 12.8% and 8.7%, respectively. We argue that the reason for the improvement is that the samples generated by Random Repeat Oversampling technique are only the copies of the original positive ones, not increasing any new information, which may cause a certain degree of overfitting ([Chawla et al., 2002](#)).

3.2 Evaluation of different feature selection methods

Our proposed new algorithm, Random Grouping strategy combined with a two-step method (Section 2), was used to select the optimal feature set. In order to explore the advantages of Random Grouping strategy and the new algorithm, we compared the performances of the four classical methods mRMR, RF, Boruta and SFS (SVM-based) with and without the strategy, and our Random Grouping strategy combined with the two-step method. The results are shown in **Table 2**.

As shown in **Table 2**, among the feature selection methods without the Random Grouping strategy, the two-step method reaches the best performance ($\text{SEN} = 0.768$, $\text{F1} = 0.591$, $\text{MCC} = 0.525$ and $\text{AUC} = 0.848$). Moreover, with the strategy considered, each method's performance has an improvement to some extent, especially in SEN, F1 and MCC scores. Thus, the Random Grouping strategy combined with a two-step algorithm, which we propose to select the optimal features for our model, performs clearly better than the other methods. We speculate the possible reason is that the two-step method considers the complementarity between features and reduces the overfitting ([Ge et al., 2016; Qiao et al., 2018](#)), and the Random Grouping strategy reduces the influence of the outlier samples on the feature selection to some extent.

Table 3. Comparison of SREPRHot with existing methods on independent testing dataset

Method	SEN	SPE	PRE	F1	MCC	AUC
XGBPRH (1.0 kcal/mol)	0.909	0.733	0.833	0.870	0.661	0.868
PrabHot (1.0 kcal/mol)	0.793	0.655	0.697	0.742	0.453	0.817
HotSPRing (1.0 kcal/mol)	0.655	0.552	0.604	0.633	0.258	0.658
SREPRHot (2.0 kcal/mol)	0.900	0.792	0.474	0.621	0.557	0.829

After the dimensional reduction by the Random Grouping strategy combined with the two-step algorithm, we finally obtained the optimal set of 18 features which are shown in **Supplementary Table S5**. Among the 18 features, nine features are sequence-based of four types (physicochemical characteristics of amino acids, PSSM, solvent exposure features and IP) and the other nine are structure-based of the other three types (DPX and CX, SASA and topological features). It should be pointed out that the residue IP proposed by us and the two topological features from the node-weighted AAN are selected as the optimal ones, which to our knowledge are used for the first time in protein–RNA hotspot prediction. IP represents the propensity of an amino acid to occur at the interface, while hotspots are a kind of special binding sites, which we think is the possible reason for the helpfulness of IP to the prediction of hotspots at binding interface. As for topological features, some studies have proved that the consideration of the node heterogeneity in network is helpful to the functional residue identification ([Yan et al., 2018](#)).

3.3 Comparison between different machine learning methods

We needed to select an appropriate machine learning method to build our model. To this aim, we compared the performances of six classic classifiers using 10-fold cross-validation on the training dataset, with the results shown in **Supplementary Table S6**. Compared with the classifiers kNN ([Cover and Hart, 1967](#)), Adaptive Boosting (Adaboost; [Freund and Schapire, 1997](#)) and eXtreme Gradient Boosting (XGBoost), GTB, RF and SVM achieve the best performances in PRE, F1 and MCC scores. In view of this, we adopted the three classifiers GTB, RF and SVM as the first-layer classifiers of our SEC, and the LR as the second layer to output the final result, which can reduce the risk of overfitting to some extent. As a result, generally SEC far outperforms the other classifiers with ACC, PRE, F1, MCC and AUC of 0.833, 0.602, 0.646, 0.581 and 0.848, respectively. Thus, the SEC is used as the machine learning classifier of SREPRHot because of its superior performance.

3.4 Performance comparison of SREPRHot with other approaches

We carried out the hotspot prediction using our method SREPRHot on the training and independent testing datasets, respectively, with the results shown in **Supplementary Table S7**. To precisely estimate SREPRHot, we repeated 10-fold cross-validation on the training dataset 50 times, obtaining ACC, SEN, F1, MCC and AUC values of 0.818 ± 0.016 , 0.814 ± 0.036 , 0.638 ± 0.022 , 0.565 ± 0.023 and 0.859 ± 0.019 , respectively. The results indicate the performances of our model are relatively stable and robust.

Additionally, we compared the performance of SREPRHot on the independent testing dataset with the existing methods PrabHot, XGBPRH and HotSPRing, and the results shown in **Table 3**. It should be pointed out that the former two were developed to predict the hotspots with a threshold of $\Delta\Delta G = 1.0$ kcal/mol, and the latter was proposed to predict the range of $\Delta\Delta G$ for a residue mutation. [Deng et al. \(2019\)](#), the developer of XGBPRH, in order to compare

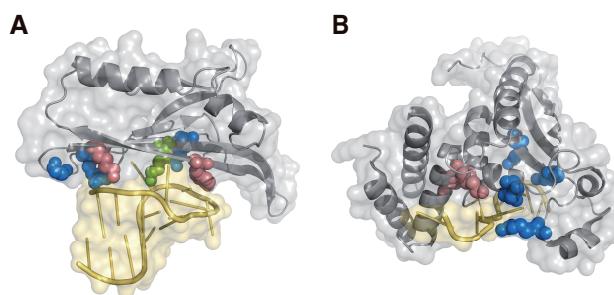


Fig. 2. Prediction results of SREPRHot on 1ZDI (A) and 4JVH (B). The predicted residues are shown in spheres. For 1ZDI, among the six predicted ones, from the left, the third (K57) and sixth (Y85) are predicted as true positives, the forth (K61) as false negative and the others (K43, R49 and S52) true negatives (A). For 4JVH, among the six predicted ones, from the left, the first (K190) and second (Q193) are predicted as true positives and the others (N97, K120, R124 and R130) as true negatives (B)

the performance of XGBPRH with HotSPRing, adopted a threshold of 1.0 kcal/mol to define hotspots for the results from HotSPRing. The results corresponding to HotSPRing in **Table 3** are from the literature ([Deng et al., 2019](#)) as HotSPRing is currently unavailable. From **Table 3**, generally XGBPRH achieves the best performance with SEN, MCC and AUC of 0.909, 0.661 and 0.868, respectively. Considering that our method uses a stricter criterion of $\Delta\Delta G \geq 2.0$ kcal/mol, SREPRHot achieves a good performance with SEN, MCC and AUC reaching 0.900, 0.557 and 0.829, respectively. The comparison indicates our approach shows a promising performance, and can be a complement to the methods with the threshold of 1.0 kcal/mol used.

3.5 Case study

As a case study, **Figure 2** shows the prediction results by SREPRHot on two protein–RNA complexes. The first is bacteriophage MS2 coat protein–RNA complex (PDB ID: 1ZDI; [Valegård et al., 1997](#)). Alanine scanning experiment gives three non-hotspots (K43A, R49A and S52A) and three hotspots (K57A, K61A and Y85A) with $\Delta\Delta G \geq 2.0$ kcal/mol. As shown in **Figure 2A**, SREPRHot identifies four non-hotspots (K43A, R49A, S52A and K61A) among which three are correctly identified. The two identified hotspots (K57A and Y85A) are all correct predictions. For the second case, which is the structure of STAR domain of Quaking protein in complex with target RNA (PDB ID: 4JVH; [Teplova et al., 2013](#)), the experiment gives four non-hotspots (N97A, K120A, R124A and R130A) and two hotspots (K190A and Q193A). Impressively, SREPRHot correctly identifies all the non-hotspots and hotspots, as shown in **Figure 2B**.

4 Conclusion

The effective prediction of binding hotspots in protein–RNA interactions is essential for understanding their specific recognition and interaction mechanisms. In this paper, a new method SREPRHot is proposed for identifying the binding hotspots, which takes the 18 features of predicted protein residues as input and gives their classification results as output. In order to deal with the data class imbalance problem caused by adopting a stricter criterion of hotspots with $\Delta\Delta G \geq 2.0$ kcal/mol, not 1.0 kcal/mol often used by the existing methods, SMOTE algorithm is utilized to generate the minority (positive) class samples to reach a data class balance. Besides conventional sequence and structural features, the two new feature types, residue IP developed by us and topological features from the node-weighted AAN, are extracted as candidate features. From them, our proposed Random Grouping feature selection strategy combined with a two-step method is utilized to pick out an optimal feature set. Finally, a stacking ensemble model is adopted, which combines three well-performing classifiers GTB, RF and SVM via

LR to construct the classification method. Compared with the existing methods, SREPRHot achieves a promising performance. We believe that our method is a new beginning in predicting binding hotspots, and in addition the strategies proposed to preprocess the data and select optimal features can also be used as a reference for future prediction works.

One thing that needs to be pointed out is that in SREPRHot performance, the protein interfacial residues need to be known. We can use the currently proposed RNA-binding residue predictors to obtain the information which include aPRBind (Liu et al., 2021a), DRNApred (Yan and Kurgan, 2017), NucBind (Su et al., 2019) and NCBRPred (Zhang et al., 2021). In addition, as for features, many tools including BioSeq-Analysis2.0 (Liu et al., 2019), BioSeq-BLM (Li et al., 2021) and DescribePROT (Zhao et al., 2021) have been developed to generate sequence- and structure-based features which can be tried to construct a powerful predictor for hotspot identification in the future.

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Data availability

All data are incorporated into the article and its online supplementary material.

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