iPPI-PseAAC(CGR): Identify protein-protein interactions by incorporating chaos game representation into PseAAC

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Highlights

- Knowledge of protein–protein interactions (PPIs) may provide valuable insights into the inner workings of cells.

- A powerful predictor has been proposed to identify PPIs in a cell.

- A user-friendly web-server for the predictor has been established by which the majority of experimental scientists can easily get their desired results.

iPPI-PseAAC(CGR): Identify protein-protein interactions by incorporating chaos game representation into PseAAC

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Running Title: Identify Protein-Protein Interactions

ABSTRACT

Investigation into the network of protein–protein interactions (PPIs) will provide valuable insights into the inner workings of cells. Accordingly, it is crucially important to develop an automated method or high-throughput tool that can efficiently predict the PPIs. In this study, a new predictor, called "iPPI-PseAAC(CGR)", was developed by incorporating the information of "chaos game representation" into the PseAAC (Pseudo Amino Acid Composition). The advantage by doing so is that some key sequence-order or sequence-pattern information can be more effectively incorporated during the treatment of the protein pair samples. The operation engine used in this predictor is the random forest algorithm. It has been observed via the cross-validations on the widely used benchmark datasets that the success rates achieved by the proposed predictor are remarkably higher than those by its existing counterparts. For the convenience of the most experimental scientists, a user-friendly web-server for the new predictor has been established at http://www.jci-bioinfo.cn/iPPI-PseAAC(CGR), by which users can easily get their desired results without the need to go through the detailed mathematics.

Keywords: Chaos game representation; Pseudo amino acid composition; Random forests; Cross validation; *Saccharomyces Cerevisiae*; *Helicobacter Pylori*; Web-server

1. INTRODUCTION

The smallest unit of life is a cell, which contains numerous protein molecules. Most of the functions critical to the cell's survival are performed via the protein-protein interactions (PPIs) therein. Therefore, it is indispensable to study PPIs in order to really understand the molecular underpinnings of life since they affect all the biological processes in a living cell.

Currently, the determination of PPIs through experiments is mainly by the three manners: (1) yeast two-hybrid assay, (2) protein chips, and (3) mass spectrometry of purified protein complexes. But it is expensive, time-consuming, and labor-intensive to determine PPIs purely based on the experimental methods. Facing the explosive growth of protein sequences occurring in the post-genomic age, we are challenged to develop computation method to identify PPIs based on the sequence information alone.

During the last decade or so, considerable efforts have been made in this regard (see, e.g., [1-8]). Although these methods did play important roles in stimulating the development of this area, further endeavor is needed to enhance the power of identifying PPIs.

The present study was initiated in an attempt to develop a new predictor called iPPI-PseAAC(CGR) to identify protein-protein interactions by using random forest algorithm [9] and incorporating "chaos game representation" [10, 11] into general PseAAC (Pseudo Amino Acid Composition) [12].

To make the presentation of this paper logically more clear and transparent, its reported results easier to be repeated by others, and its proposed method practically more useful, the 5-step rules [12] were followed, as done in a series of recent publications (see, e.g., [13-38]).

2. MATERIAL AND METHODS

2.1. Benchmark Datasets

The first step in the 5-step rules [12] is how to construct or select a valid benchmark dataset to train and test the predictor. Two benchmark datasets were used for the current study: one is called the S.C. dataset used for studying the PPIs in the cell of *Saccharomyces Cerevisiae*; while the other called the H.P. dataset for studying the PPIs in the cell of *Helicobacter Pylori*.

2.1.1. S.C. dataset. To obtain a high quality benchmark dataset, the proteins in *Saccharomyces Cerevisiae* [39] were collected according to the following criteria. (**1**) To avoid fragments, each of the included proteins must contain at least 50 residues. (**2**) To reduce the homology bias, none of the included proteins has ^{3} 40% pairwise sequence identity. Based on the 7,374 proteins thus obtained, the S.C. dataset, $\mathbb{S}_{\text{S.C.}}$, was constructed as formulated below

$$\mathbb{S}_{\text{S.C.}} = \mathbb{S}^{+}_{\text{S.C.}} \cup \mathbb{S}^{-}_{\text{S.C.}} \tag{1}$$

where $\mathbb{S}_{\text{S.C.}}$ contains 50,652 protein pairs, of which 17,505 are interactive belonging to the positive subset $\mathbb{S}^{+}_{\text{S.C.}}$, while 33,147 are non-interactive belonging to the negative subset $\mathbb{S}^{-}_{\text{S.C.}}$, and ∪ represents the union in the set theory. For the codes of these proteins and their detailed sequences, see Supporting Information S1.

2.1.2. H.P. dataset. For facilitating comparison later, the benchmark dataset for studying the PPIs in the cell of *Helicobacter Pylori* [40] was also considered since it was used by many investigators [40-45] to test their methods with the results well documented. Likewise, here such benchmark is formulated by

$$\mathbb{S}_{\text{H.P.}} = \mathbb{S}^{+}_{\text{H.P.}} \cup \mathbb{S}^{-}_{\text{H.P.}} \tag{2}$$

where $\mathbb{S}_{\text{H.P.}}$ contains 2,916 protein pairs, of which 1,458 are interactive belonging to the positive subset $\mathbb{S}^{+}_{\text{H.P.}}$, while 1,458 are non-interactive belonging to the negative subset $\mathbb{S}^{-}$. For the codes of these proteins and their detailed sequences, see Supporting Information S2.

2.2. Using Pseudo Amino Acid Composition to Represent Protein Pairs

The second step in the 5-step rules [12] is how to formulate the biological sequence samples with a discrete model or a vector, yet still keep considerable sequence-order information or key pattern characteristic. This is because all the existing machine-learning algorithms (such as “Covariance Discriminant” or “CD” algorithm [46, 47], “Nearest Neighbor” or “NN” algorithm [48, 49], “Support Vector Machine” or “SVM” algorithm [50, 51], and “Random Forest” or “RF” algorithm [52, 53]) can only handle vectors as elaborated in a comprehensive review [54]. However, a vector defined in a discrete model may completely lose all the sequence-pattern information. To avoid completely losing the sequence-pattern information for proteins, the pseudo amino acid composition [55] or PseAAC [56] was proposed. Ever since the concept of Chou’s PseAAC was proposed, it has been widely used in nearly all the areas of computational proteomics (see, e.g., [57-63] [13, 50, 64-95] as well as a long list of references cited in [96]). Because it has been widely and increasingly used, recently three powerful open access soft-wares, called ‘PseAAC-Builder’ [97], ‘propy’ [98], and ‘PseAAC-General’ [99], were established: the former two are for generating various modes of Chou’s special PseAAC [100]; while the 3rd one for those of Chou’s general PseAAC [12], including not only all the special modes of feature vectors for proteins but also the higher level feature vectors such as “Functional Domain” or “FunD” mode, “Gene Ontology” or “GO” mode, and “Sequential Evolution” or “PSSM” mode. Encouraged by the successes of using PseAAC to deal with protein/peptide sequences, its idea and approach were extended to PseKNC (Pseudo K-tuple Nucleotide Composition) to generate various feature vectors for DNA/RNA sequences [101] that have proved very successful as well [15, 17, 19, 102-106]. Particularly, recently a very powerful web-server called ‘Pse-in-One’ [107] and its updated version ‘Pse-in-One2.0’ [108] have been established that can be used to generate any desired feature vectors for protein/peptide and DNA/RNA sequences according to the users’ need or their own definition.” According to the general PseAAC [12], any protein sequence can be formulated as a PseAAC vector given by

$$\mathbf{P} = [\Psi_1 \quad \Psi_2 \quad \cdots \quad \Psi_u \quad \cdots \quad \Psi_\Omega]^{\mathbf{T}} \tag{3}$$

where **T** is a transpose operator, while the subscript Ω is an integer parameter and its value as well as the components Ψ_u $(u = 1, 2, \cdots, \Omega)$ will depend on how to extract the desired information from the amino acid sequence of **P**, as elaborated below.

In this study, we are to use CGR (Chaos Game Representation) proposed by Fiser et al. [10] to define the pseudo components in Eq.3. To realize this, we first converted the protein sequences to the nucleotide sequences according to **Table 1**, which was proposed by Deschavanne and Tuffery [109]. The advantage of using such code-converting method is able to keep balanced base composition so as to maximize the differences between the amino acid codes. Particularly, it can also provide useful insights via intuitive plot or graph for facilitating analysis of complicated biological systems as demonstrated in a series of works by the previous investigators (see, e.g. [110-125]).

A brief description to generate the CGR via **Table 1** is as follows. In a [0,1]×[0,1] square, the four vertices of the defined square correspond to the four letters: **A**, **C**, **G**, and **T**. Then, the CGR-plot can be obtained via the following steps: (**1**) put an initial point at an random site of the square (we selected the center of it in this paper); (**2**) place the second point at the half way between the initial point and the vertex corresponding to the first letter of the nucleotide sequence; (**3**) place the *i*th point half way between the (*i*-1)th point and the vertex corresponding to the *i*th letter; (**4**) go to Step 3 until you reach the end of the nucleotide sequence.

Shown in **Fig.1** is the CGR thus generated for a given protein sequence. As we can see from the figure, many important features, which are hidden in a long and complicated biological sequence, can be clearly revealed via its CGR. Subsequently, we divided the GGR square to 4×4=16 sub-squares with each having the same size. By calculating the number of the points within each of the 16 sub-squares, plus the occurrence frequencies of the 20 native amino acids therein, we have a total of $(16 + 20) = 36$ components to define the components of Eq.3 and its Ω. In other words, the PseAAC vector for **P** in this study is a 36-D (dimensional) vector.

Accordingly, for a protein pair formed by $\mathbf{P}^{k1}$ and $\mathbf{P}^{k2}$, the corresponding PseAAC vector can be formulated by their orthogonal sum; i.e.,

$$\mathbf{P}^{k1} \oplus \mathbf{P}^{k2} = [\Psi_1^{k1} \quad \Psi_2^{k1} \quad \cdots \quad \Psi_{36}^{k1} \quad \Psi_1^{k2} \cdots \quad \Psi_{36}^{k2}]^{\mathbf{T}} \tag{4}$$

where $\mathbf{P}^{k1}$ and $\mathbf{P}^{k2}$ as well as their components have exactly the same meaning as those in Eq.3 except for that they are now referred to the $\mathbf{P}^{k1}$ or $\mathbf{P}^{k2}$, instead of **P**, and the symbol $\oplus$ represents the sign of orthogonal sum. Thus, a 72-D PseAAC vector is used to formulate the sample of a PP pair.

2.3. Random Forest and Ensemble Classifier

The third step in the 5-step rules [12] is how to introduce or develop a powerful algorithm (or engine) to operate the prediction. Here we used the random forest (RF) algorithm [9], which has been widely used in the area of computational biology (see, e.g., [7, 33, 52, 53, 106, 126-134]). The detailed procedures and formulation of RF have been very clearly described in [9], and hence there is no need to repeat here. The random forests algorithm usually produces a remarkable improvement in performance over the single decision tree classifier [135]. Moreover, we found that the random forests algorithm was not sensitive to the number of trees according to the aforementioned sample formulation. Thus, a total of 200 trees were used in order to alleviate computational cost and the over-fitting problem.

In this study, there are two benchmark datasets, one is the S.C. dataset (see Eq.1 and Supporting Information S1) and the other is the H.P. datasets (see Eq.2 and Supporting Information S2). The model trained by the former is for predicting the PPIs in *Saccharomyces Cerevisiae*; while the model trained by the latter is for predicting the PPIs in *Helicobacter Pylori*. In the S.C. dataset, the number of negative samples is much larger than that of the positive samples, meaning it is a very imbalanced or skewed dataset. But in the H.P. dataset there is no such a problem since its positive subset is the same in size as its negative subset. To alleviate the biased outcomes caused by the skewed dataset, there are some existing methods to add some theoretical or hypothetical samples into the smaller subsets, such as the “Monte Carlo samples expanding” approach [136, 137], “seed-propagation” approach [138], “LogiBoost” [139], “SMOTE” (synthetic minority over-sampling technique) approach [140-142], “Bootstrap” approach [8], and IHTS (Inserting Hypothetical Training Samples) treatment [36, 143-147]. But here we used a different approach to deal with the unbalanced problem, as described below.

From the 33,147(=$n_{\text{S.C.}}^{-}$) negative samples in $\mathbb{S}_{\text{S.C.}}^{-}$ of Eq.1, we randomly picked 17,505 (=$n_{\text{S.C.}}^{+}$) samples to form a negative sub-subset whose size is the same as the positive subset $\mathbb{S}_{\text{S.C.}}^{+}$. Repeat the above procedure for 7 times, generating an array of 7 negative sub-subsets. Using each of these negative sub-subsets and the positive subset $\mathbb{S}_{\text{S.C.}}^{+}$ (Eq.1) to train the Random Forest model, we obtained an array of 7 individual predictors $\text{RF}(k)$ $(k = 1,2,\cdots,7)$. Based on them, an ensemble classifier was formed via a voting system, as formulated by

$$\mathbb{RF}^{\text{E}} = \text{RF}(1)\forall\cdots\forall\text{RF}(7) = \forall_{k=1}^{7}\text{RF}(k) \tag{5}$$

where $\mathbb{RF}^{\text{E}}$ stands for the ensemble classifier, and the symbol ∀ for the fusing operator. For the detailed procedures of how to fuse the results from the seven individual predictors to reach a final outcome via the voting system, see Eqs.30-35 in [148], where a crystal clear and elegant derivation was elaborated and hence there is no need to repeat here. To provide an intuitive picture, a flowchart is given in **Fig.2** to illustrate how the seven individual RF predictors are fused into the ensemble classifier.

But for predicting the PPIs in the *Helicobacter Pylor*, there is no need at all to go through the above ensemble-learning procedure. This is because the H.P. benchmark dataset is a balanced one; its negative and positive subsets are the same in size, namely $n_{\text{H.P.}}^{-} = n_{\text{H.P.}}^{+}$. Therefore, we can directly use $\mathbb{S}_{\text{H.P.}}$(Eq.2) to train the random forest model.

The final predictor thus obtained is called “**iPPI-PseAAC(CGR)**”, where “i” stands for “identify”, “PPI” for “protein-protein interaction”, and “PseAAC(CGR)” for “incorporating CGR (Chaos Game Representation) into general PseAAC (Pseudo Amino Acid Composition)”. In dealing with the *Saccharomyces Cerevisiae* system, the RF-ensemble engine is on; in dealing with the *Helicobacter Pylor* system, however, the RF-ensemble engine will be replaced by RF only.

2.4. Cross-Validation

The fourth step in the 5-step rules [12] is how to properly perform cross-validation tests to objectively evaluate the anticipated accuracy of the predictor. To address this problem, we need to consider the following two sub-problems. (**1**) What metrics should be used to quantitatively measure the predictor's quality? (**2**) What test approach should be adopted to score the metrics?

2.4.1. A set of four intuitive metrics

For examining the performance of a predictor in identifying whether two proteins are in interaction with each other, four metrics [149] are often used in literature; they are (**1**) overall accuracy or Acc, (**2**) Mathew's correlation coefficient or MCC, (**3**) sensitivity or Sn, and (**4**) specificity or Sp. But their conventional formulations directly copied from math books are difficult to understand for most experimental scientists, particularly the one for MCC. Fortunately, by using the symbols introduced by Chou [150, 151] in studying the signal peptide cleavage sites, a set of intuitive metrics were derived [51, 152, 153], as given below

$$\begin{cases} \mathrm{Sn} = 1 - \dfrac{N_-^+}{N^+} & 0 \ \leq \mathrm{Sn} \leq 1 \\ \mathrm{Sp} = 1 - \dfrac{N_+^-}{N^-} & 0 \leq \mathrm{Sp} \leq 1 \\ \mathrm{Acc} = 1 - \dfrac{N_-^+ + N_+^-}{N^+ + N^-} & 0 \leq \mathrm{Acc} \leq 1 \\ \mathrm{MCC} = \dfrac{1 - \left(\dfrac{N_-^+}{N^+} + \dfrac{N_+^-}{N^-}\right)}{\sqrt{\left(1 + \dfrac{N_+^- - N_-^+}{N^+}\right)\left(1 + \dfrac{N_-^+ - N_+^-}{N^-}\right)}} & -1 \leq \mathrm{MCC} \leq 1 \end{cases} \tag{5}$$

where N^+ represents the total number of interactive protein pairs investigated whereas N_-^+ the number of true interactive pairs incorrectly predicted as of non-interactive pair; N^- the total number of the non-interactive protein pairs investigated whereas N_+^- the number of non-interactive protein pairs incorrectly predicted as of interactive pair. Because of its intuitiveness and ease to be understood, the set of metrics has been increasingly and widely used in computational biology (see, e.g., [8, 15, 17, 19, 20, 22, 24-26, 28-31, 33, 35, 53, 72, 102, 104-106, 129, 130, 132-134, 141-144, 154-179]. It is instructive to point out that both the original four metrics [149] in math books and the intuitive ones in Eq.5 are valid only for the single-label systems (where each sample belongs to one and only one class or attribute). For the multi-label systems (where a sample may simultaneously belong to several classes or attributes), whose existence has become more frequent in system biology [14, 16, 18, 23, 32, 34, 36, 145, 180], system medicine [21, 181] and biomedicine [131], a completely different set of metrics as defined in [182] is absolutely needed.

2.4.2. Cross-validation

In statistical prediction, the following three cross-validation methods are often used to check the performance of a predictor: independent dataset test, subsampling (or K-fold cross-validation) test, and jackknife test [183]. Of the three test methods, the jackknife test is deemed the least arbitrary that can always yield a unique result for a given benchmark dataset as elaborated in [12] and demonstrated by Eqs.28-30 therein. However, to reduce the computational time, in this study we adopted the 5-fold and 10-fold cross validation tests, as done by many investigators with random forests algorithm as the operation engine.

2.5. Web-Server for iPPI-PseAAC(CGR)

The last but not the least important step of the 5-step rules [12] is how to establish a user-friendly web-server for the predictor that is accessible to the public. As pointed out in [184] and demonstrated in a series of recent publications (see, e.g., [14-21, 23, 26, 27, 29, 32, 34, 105, 134, 164, 174, 175, 177, 178, 180, 185-188]), user-friendly and publicly accessible web-servers represent the future direction for developing practically more useful prediction methods and computational tools. Actually, many practically useful web-servers have significantly increased the impacts of bioinformatics on medical science [54], driving medicinal chemistry into an unprecedented revolution [96]. In view of this, the web-server for the IPP-PseAAC (CGR) predictor has also been established at http://www.jci-bioinfo.cn/iPPI-PseAAC(CGR). Shown in **Fig.3** is the semi-screenshot of its top page. Note: when using the web-server, click the S.C. button if you want to predict the PPIs in *Saccharomyces Cerevisiae*; click on H.P. button if you want to predict the PPIs in *Helicobacter Pylori*.

3. RESULTS AND DISCUSSION

Listed in **Table 2** are the scores of the four metrics (cf. Eq.5) obtained by iPPI-PseAAC(CGR) on the S.C. benchmark dataset (Supporting Information S1) via the 5-fold cross-validation. For facilitating comparison, listed there are also the corresponding rates obtained by the existing state-of-the-art method [7]. As we can see from the table that, the success rates for Acc and MCC achieved by the proposed predictor iPPI-PseAAC(CGR) are higher than those by iPPI-Esml [7], the existing state-of-the-art predictor. Although the rate of Sn by iPPI-Esml is about 6% higher than that by iPPI-PseAAC(CGR), the rate of Sp by iPPI-Esml is about 8% lower than that by the iPPI-PseAAC(CGR). Actually, among the four metrics in Eq.5, the most important are the Acc and MCC: the former reflects the overall accuracy of a predictor; while the latte, its stability in practical applications. Sn and Sp are actually constrained with each other [137]. Therefore, it is meaningless to use only one of the two for comparing the quality of two predictors. In other words, a meaningful comparison in this regard should use the rates of both Sn and Sp, or even better use their combination that is none but MCC, for which the rate achieved by the iPPI-PseAAC(CGR) is about 6% higher than that by the iPPI-Esml predictor.

Listed in **Table 3** are the success rates obtained by the proposed predictor on the H.P. benchmark dataset (Supporting Information S2) via the 10-fold cross-validation. For facilitating comparison, listed there are also the corresponding rates obtained by the other six prediction methods [40-45]. It is clearly shown from there that the newly proposed predictor iPPI-PseAAC(CGR) is remarkably superior to its six cohorts. Besides, none of them [40-45] has web-server as iPPI-PseAAC(CGR) does, and hence their practical application value by the majority of experimental scientists is very limited.

4. CONCLUSION

iPPI-PseAAC(CGR) is a powerful predictor for identifying the protein-protein interactions in cell according to the protein sequence information alone. In the predictor, each protein is formulated by a PseAAC vector formed by 36 components, of which 20 are the occurrence frequencies of the 20 native amino acid residues in the protein, and the remaining 16 components are derived from the chaos game representation. Thus, each protein pair is denoted by a $36 \times 2 = 72$-D PseAAC vector. The learning machine implemented in the new predictor is random forests and their ensemble. Its success rates have been examined by two stringent benchmark datasets: one for *Saccharomyces Cerevisiae*, and one for *Helicobacter Pylori*, indicating the new predictor is superior to its counterparts. A public-accessible web-server for iPPI-PseAAC(CGR) has been established. We anticipate that it will become a very useful high throughput toll for identifying PPIs in any related areas.

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Table 1. Reverse encoding for the amino acids used in this study

A=GCT	G=GGT	M=ATG	S=TCA	C=TGC	H=CAC	N=AAC	T=ACT	D=GAC	I=ATT
P=CCA	V=GTG	E=GAG	K=AAG	Q=CAG	W=TGG	F=TTC	L=CTA	R=CGA	Y=TAC

The encoding approach was according to Deschavanne and Tuffery [109].

Table 2. The results obtained by the 5-fold cross-validation on the S.C. benchmark dataset (Online Supporting Information S1).

Method	Acc (%)[a]	MCC [a]	Sn (%)[a]	Sp (%)[a]
iPPI-Esml[b]	84.29	0.7063	97.64	77.43
iPPI-PseAAC(CGR)[c]	88.01	0.7624	91.09	85.37

[a] See Eq.5 for the metrics definition.
[b] Proposed in [7].
[c]Proposed in this paper.

Table 3. Compared with the other seven methods via the 10-cross-validation on the *H. P.* dataset [40] (Online Supporting Information S2).

Method	Acc (%)	MCC	Sn (%)	Sp (%)	Web-server
Bock and Gough [a]	75.80	N/A	69.80	80.20	No
Guo et al. [b]	80.96	0.5577	78.65	83.20	No
Martin [c]	83.40	N/A	79.90	85.70	No
Nanni [d]	83.00	N/A	80.60	85.10	No
Nanni and Lumini [e]	86.60	N/A	86.70	85.00	No
Xia et al. [f]	88.40	N/A	88.20	89.20	No
iPPI-PseAAC(CGR)[g]	**92.95**	**0.8505**	**97.61**	**88.00**	**Yes**

[a] Results reported by Bock et al. [41].
[b] Results reported by Guo et al. [42].
[c] Results reported by Martin et al. [40].
[d] Results reported by Nanni. [43].
[e] Results reported by Nanni et al. [44].
[f] Results reported by Xia et al. [45].
[g] Proposed in this paper.

FIGURE LEGENDS

Figure 1. A CGR-plot for one protein sequence. See the text in Section 2.2 for further explanation.

Figure 2. The flowchart to show the procedure of the ensemble approach. See the text in Section 2.3 for further explanation.

Figure3. A semi-screenshot to show the top-page of the iPPI-PseAAC(GPR) web-server at http://www.jci-bioinfo.cn/iPPI-PseAAC(CGR). See the text in Section 2.5 for further explanation.

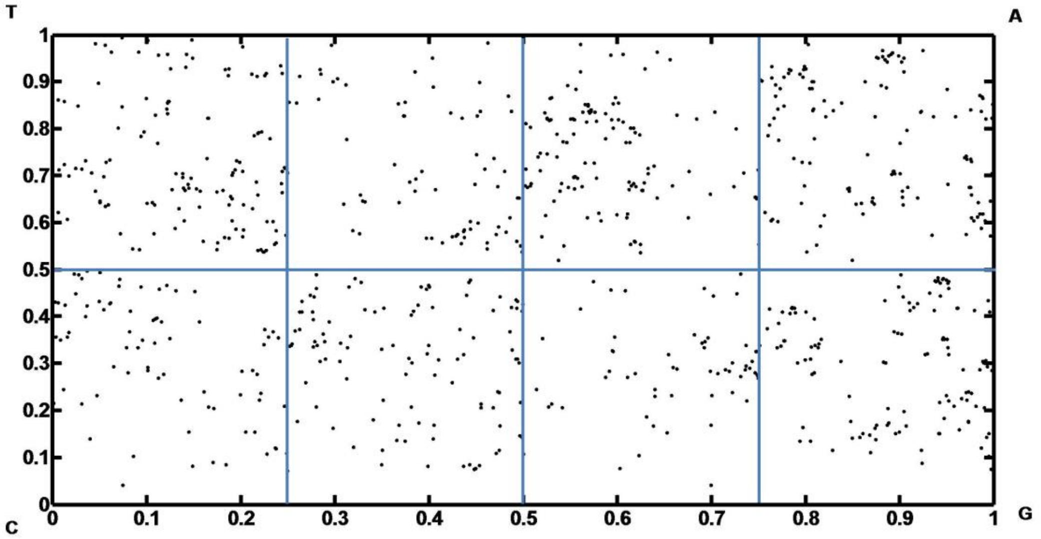


Figure 1

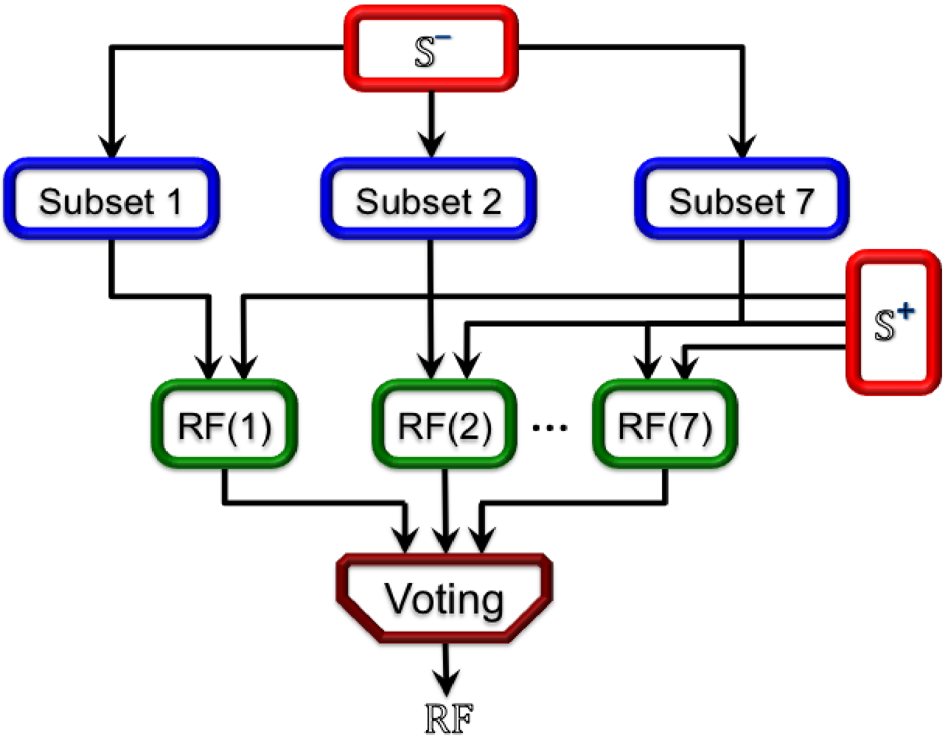
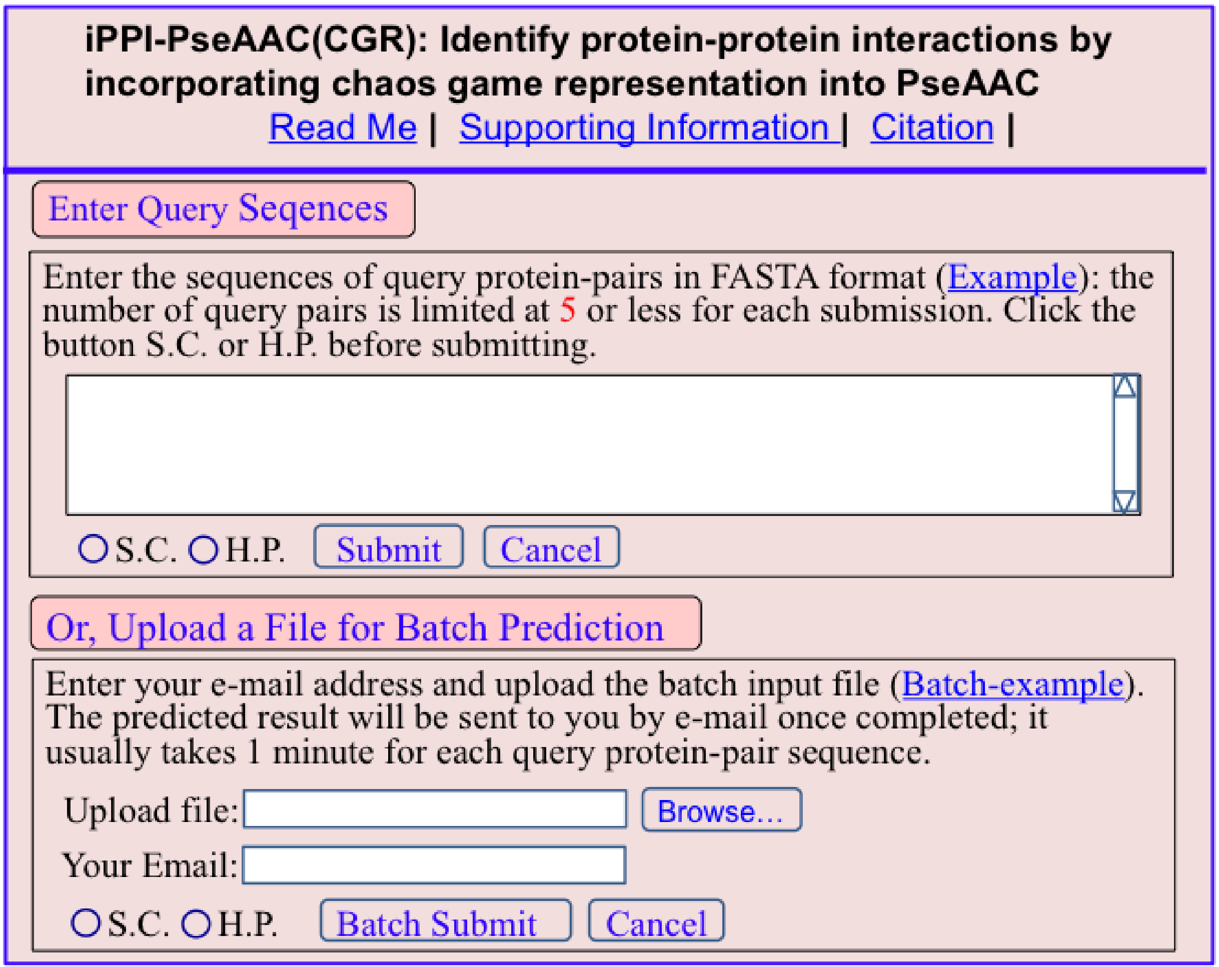


Figure 2



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Enter Query Seqences

Enter the sequences of query protein-pairs in FASTA format (Example): the number of query pairs is limited at 5 or less for each submission. Click the button S.C. or H.P. before submitting.

Or, Upload a File for Batch Prediction

Enter your e-mail address and upload the batch input file (Batch-example). The predicted result will be sent to you by e-mail once completed; it usually takes 1 minute for each query protein-pair sequence.

Upload file:

Your Email:

Figure 3

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