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iPPI-Esml: an ensemble classifier for identifying the interactions of proteins by incorporating their physicochemical properties and wavelet transforms into PseAAC

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Running Title: Identification of Protein-Protein Interactions

ABSTRACT

A cell contains thousands of proteins. Many important functions of cell are carried out through the proteins therein. Proteins rarely function alone. Most of their functions essential to life are associated with various types of protein-protein interactions (PPIs). Therefore, knowledge of PPIs is fundamental for both basic research and drug development. With the avalanche of proteins sequences generated in the postgenomic age, it is highly desired to develop computational methods for timely acquiring this kind of knowledge. Here, a new predictor, called "iPPI-Emsl", is developed. In the predictor, a protein sample is formulated by incorporating the following two types of information into the general form of PseAAC (pseudo amino acid composition): (1) the physicochemical properties derived from the constituent amino acids of a protein; (2) the wavelet transforms derived from the numerical series along a protein chain. The operation engine to run the predictor is an ensemble classifier formed by fusing seven individual random forest engines via a voting

system. It is demonstrated with the benchmark dataset from *S. cerevisiae* as well as the dataset from *H. pylori* that the new predictor achieves remarkably higher success rates than any of the existing predictors in this area. The new predictor' web-server has been established at http://www.jci-bioinfo.cn/iPPI-Esml. For the convenience of most experimental scientists, we have further provided a step-by-step guide, by which users can easily get their desired results without the need to follow the complicated mathematics involved during its development.

Keywords: Physicochemical properties; Wavelets transforms; Pseudo amino acid composition; Random Forests; Fusion; Voting system; Ensemble classifier

I. INTRODUCTION

Proteins play a vital role in nearly all biology functions such as composing cellular structure and promoting chemical reactions. Proteins in a cell rarely function in isolation but are actively and selectively interacting with each other (**Fig.1**). Most of their functions essential to life are associated with different types of protein-protein interactions (PPIs). For instance: proteins are modified and degraded by enzyme proteins; many marvelous biological functions in proteins, such as allosteric regulation, are realized via the interactions between the protein subunits (Chou, 1989b; Perutz, 1942); signal transmission between cells is via binding of protein messengers to protein receptors (Chou, 2005a); proteins are directed to the correct compartments of cells thru binding to other proteins; structural connections between cells are established via PPIs; the molecular mechanism of muscle contraction as well as the opening and closing of ion-channels are also closely associated with PPIs (Huang et al., 2008; OuYang et al., 2013 ; Schnell and Chou, 2008).

On the other hand, PPIs are also related to various disease states. For instance, if a cell suddenly lost some normal or necessary PPIs, the deprived cell would become "blind" and "deaf", completely paralytic finally leading to perish. Also, if many abnormal or unwanted PPIs suddenly occurred in a cell, the "unfortunate" cell would completely lose control, leading to network confuse and a terrible disaster.

Accordingly, it is vitally important to characterize PPIs and understand their interaction network, an important subject in the discipline called "protein network" or a frontier for investigating the functional relationship of proteins in a cell. However, it is by no means an easy job due to the extreme complexity of the problem concerned. As is well known, using graphical approaches to study complicated biological problems can provide an intuitive picture or useful insights for helping analyzing complicated relations in these systems, as demonstrated by many previous studies on a series of important biological topics, such as enzyme-catalyzed reactions (Zhou and Deng, 1984), inhibition of HIV-1 reverse transcriptase (Althaus et al., 1993a; Althaus et al., 1993b), protein folding kinetics (Chou, 1990), and using wenxiang diagram or graph (Chou et al., 2011) to study protein-protein

interactions (Zhou, 2011a; Zhou, 2011b; Zhou and Huang, 2013). In view of this, we are also using the vertex-arc graph (**Fig.1**) to express a protein-protein interaction network, where the vertex denotes each of the proteins in the network system while the arc to indicate their relation. As we can see from the figure, the PPI systems are indeed very complicated. Therefore, it is absolutely necessary to combine the experimental and computational approaches together for really understanding this kind of systems.

During the last decade or so, various experimental techniques have been developed for determining PPIs, such as yeast two-hybrid systems (Fields and Song, 1989; Ito et al., 2001), mass spectrometry (Gavin et al., 2002), and protein chip (Zhu et al., 2001). But only very small portion of PPI's were identified (Han et al., 2005) because it was time-consuming, labor-intensive and expensive by using experimental technique alone.

Fortunately, the success of the human genome project has provided us with a significant amount of useful data to conduct statistical analyses in this regard, and hence made it feasible to predict the PPIs by computational approaches.

Our rationale is as follows. It is virtually axiomatic that the amino acid sequence of a protein will determine its 3D (three-dimensional) structure (Anfinsen, 1973); while the latter will determine its biological function. Accordingly, the sequence information alone of proteins can certainly determine their interaction relationship. Actually, many computational methods have been proposed in this regard (Chou and Cai, 2006; Espadaler et al., 2005; Gomez et al., 2003; Guo et al., 2008b; Marcotte et al., 1999; Shen et al., 2007b; Xia et al., 2010a; Xia et al., 2010b; Yang et al., 2010). Each of these methods has its own merit, and did play a role in stimulating the development of this area. However, all the aforementioned methods were based on a single learner without using the ensemble learning technique, and hence their power might be limited. Besides, in the aforementioned methods, none of physicochemical properties was taken into account, which might further limit the prediction quality.

Many evidences have indicated that using ensemble classifier can significantly enhance the success rates in recognizing protein fold pattern (Shen and Chou, 2006), identifying membrane protein types (Shen and Chou, 2007), and predicting protein subcellular localization (see, e.g., (Chou and Shen, 2006a; Shen et al., 2007a)). In other words, in comparison with a single classifier, the ensemble classifier formed by fusing multiple single classifiers can achieve much better prediction quality with more generalized ability (Chou and Shen, 2007a; Jia et al., 2011).

Stimulated by the successes of using ensemble classifiers for predicting protein attributes (Chou and Shen, 2006a; Shen and Chou, 2006), Nanni et al. (Nanni and Lumini, 2006) developed an ensemble classifier by fusing K-local hyperplanes for predicting PPIs, remarkably enhancing the success rate. Unfortunately, for the prediction method (Nanni and Lumini, 2006), no web-server whatsoever has been established. Therefore, its practical application value is considerably limited, particularly for most experimental scientists.

The present study was aimed at (1) developing a new and more powerful ensemble classifier by incorporating the physicochemical properties concerned, and (2) establishing a user-friendly web-server for the new PPI predictor.

As reflected by a number of recent articles (Chen et al., 2013; Chen et al., 2014a; Chen et al., 2014b; Guo et al., 2014; Lin et al., 2014; Liu et al., 2015b; Liu et al., 2014a; Liu et al., 2014b; Xu et al., 2014b) in response to the call (Chou, 2011), in presenting a sequence-based statistical predictor for a biological system, one should make the following five procedures very clear: (1) how to construct or select a valid benchmark dataset to train and test the predictor; (2) how to formulate the biological sequence samples with an effective mathematical expression that can truly reflect their intrinsic correlation with the target to be predicted; (3) how to introduce or develop a powerful algorithm (or engine) to operate the prediction; (4) how to properly perform cross-validation tests to objectively evaluate its anticipated accuracy; (5) how to establish a user-friendly web-server that is accessible to the public. Below, we are to address the five procedures one-by-one.

II. MATERIAL AND METHODS

II.1. Benchmark Datasets

Two benchmark datasets were used for the current study. One is called the S.C. dataset used to study the PPIs in the cell of *Saccharomyces Cerevisiae*, while the other called the H.P. dataset used to study the PPIs in the cell of *Helicobacter Pylori*.

S.C. Dataset. To obtain a high quality benchmark dataset, the source *Saccharomyces Cerevisiae* proteins for the S.C. dataset were collected according to the following criteria: (1) each of the included proteins must contain at least 50 residues in order to avoid fragments (Chou and Shen, 2007a); (2) none of the included proteins has $\geq 40\%$ pairwise sequence identity to any other in order to reduce the homology bias. From the 7,374 source proteins thus obtained and using DIP (Database of Interacting Proteins) (Xenarios et al., 2002), we can obtain 17,505 interactive protein pairs. As for the non-interacting pairs that are not readily available from DIP database, we constructed them as follows. The non-interactive pairs were generated based on such an assumption that proteins located at different subcellular localizations do not interact with each other (Guo et al., 2008a; Shen et al., 2007b). The subcellular location information of the proteins concerned was extracted from Swiss-Prot (http://www.expasy.org/sprot/) by considering the following seven locations: Cytoplasm, Nucleus, Mitochondrion, Endoplasmic Reticulum, Golgi Apparatus, Peroxisome, Vacuole. Subsequently, the negative data were formed via pairing the proteins concerned from one location site with those from a different one. The following requirement must be satisfied when doing so (Guo et al., 2008a; Shen et al., 2007b): the non-interacting pairs thus formed should not also occur in the positive dataset of interactive pairs. A total of 5,943 negative pairs were thus generated. As pointed out by the authors in (Ben-Hur and Noble, 2006), however, the restricting negative samples formed from different subcellular locations may lead to a biased estimate of the accuracy for a PPI predictor. Therefore, it is necessary to also generate the negative samples from the same subcellular location to reduce this kind of bias. In view of this, additional negative samples were generated according to the principle that the protein pairs at the same localization were considered as the negative pairs if none of them has occurred in the yeast positive pairs. Thus, additional 27,204 negative pairs were generated within each of the aforementioned seven subcellular locations: 8,000 within Cytoplasm, 8,000 within Nucleus, 8,284 within Mitochondrion, 1,953 within Endoplasmic Reticulum, 300 within Golgi apparatus, 171 within Peroxisome,

and 496 within Vacuole. Finally, the benchmark dataset thus established can be formulated below

$$\mathbb{S}_{\text{S.C.}} = \mathbb{S}^{+}_{\text{S.C.}} \bigcup \mathbb{S}^{-}_{\text{S.C.}} \quad (1)$$

where $\mathbb{S}_{\text{S.C.}}$ is the *S.C.* benchmark dataset for *Saccharomyces Cerevisiae* that contains 50,652 protein pairs, of which 17,505 are interactive pairs belonging to the positive subset $\mathbb{S}^{+}_{\text{S.C.}}$, $5943 + 27204 = 33{,}147$ are non-interactive pairs belonging to the negative subset $\mathbb{S}^{-}_{\text{S.C.}}$, and $\bigcup$ represents the union in the set theory. For the details of these protein pairs and their DIP codes, see Online Supporting Information S1.

H.P. Dataset. For facilitating comparison later, the benchmark dataset used to study the PPIs in the cell of *Helicobacter Pylori* was taken from (Martin et al., 2005) since many investigators used it to test their own methods with the success rates well documented (see, e.g., (Nanni, 2005; Nanni and Lumini, 2006; Xia et al., 2010b)). Likewise, the H.P. dataset can be formulated as

$$\mathbb{S}_{\text{H.P.}} = \mathbb{S}^{+}_{\text{H.P.}} \bigcup \mathbb{S}^{-}_{\text{H.P.}} \quad (2)$$

where $\mathbb{S}_{\text{H.P.}}$ contains 2,916 *Helicobacter Pylori* protein pairs, $\mathbb{S}^{+}_{\text{H.P.}}$ is the positive subset containing 1,458 interactive protein pairs, and $\mathbb{S}^{-}_{\text{H.P.}}$ is the negative subset containing 1,458 non-interactive protein pairs. For the details of these protein pairs and their corresponding protein sequences, see Online Supporting Information S2 and Online Supporting Information S3, respectively.

II.2. Using Pseudo Amino Acid Composition to Represent Protein Pairs

One of the most challenging problems in computational biology today is how to effectively formulate the sequence of a biological sample (such as protein, peptide, DNA, or RNA) with a discrete model or a vector that can considerably keep its sequence order information or capture its key features. The reasons are as follows. (1) If using the sequential model, i.e., the model in which all the samples are represented by their original sequences, it is hardly able to train a machine that can cover all the possible cases concerned, as elaborated in (Chou, 2011). (2) All the existing computational algorithms, such as optimization approach (Zhang and Chou, 1992), correlation-angle approach (Chou, 1993), covariance discriminant (CD) (Chen et al., 2012), neural network (Feng et al., 2005), SLLE algorithm (Wang et al., 2005), support vector machine (SVM) (Lin et al., 2014; Xu et al., 2014a), random forest (Lin et al., 2011), conditional random field (Xu et al., 2013a), nearest neighbor (NN) (Cai and Chou, 2003), K-nearest neighbor (KNN) (Shen et al., 2006), OET-KNN (Shen and Chou, 2009a), Fuzzy K-nearest neighbor (Xiao et al., 2013a), and ML-KNN algorithm (Xiao et al., 2011), can only handle vector but not sequence samples.

However, a vector defined in a discrete model may completely lose the sequence-order

information. To cope with such a dilemma, the approach of pseudo amino acid composition (Chou, 2001a; Chou, 2005b) or Chou's PseAAC (Cao et al., 2013; Du et al., 2012; Lin and Lapointe, 2013) was proposed. Ever since it was introduced in 2001 (Chou, 2001a), the concept of PseAAC has been widely used in almost all the areas of computational proteomics, such as in predicting antifreeze proteins (Mondal and Pai, 2014), predicting protein structural class (Kong et al., 2014; Zhang et al., 2014b), predicting anticancer peptides (Hajisharifi et al., 2014), identifying bacterial virulent proteins (Nanni et al., 2012b), predicting protein subcellular location in various organisms and levels (Kandaswamy et al., 2010; Li et al., 2014; Mei, 2012; Nanni and Lumini, 2008; Zhang et al., 2008; Zuo et al., 2014), predicting membrane protein types (Chen and Li, 2013; Han et al., 2014), discriminating outer membrane proteins (Hayat and Khan, 2012), analyzing genetic sequence (Georgiou et al., 2013), identifying cyclin proteins (Mohabatkar, 2010), predicting GABA(A) receptor proteins (Mohabatkar et al., 2011), identifying antibacterial peptides (Khosravian et al., 2013), identifying allergenic proteins (Mohabatkar et al., 2013), predicting metalloproteinase family (Mohammad Beigi et al., 2011), identifying GPCRs and their types (Zia Ur and Khan, 2012), identifying the types of conotoxins (Ding et al., 2014), identifying protein quaternary structural attributes (Sun et al., 2012), identifying risk type of human papillomaviruses (Esmaeili et al., 2010), identifying various PTM (post-translational modification) sites in proteins (Jia et al., 2014; Qiu et al., 2014b; Qiu et al., 2014c; Xu et al., 2013a; Xu et al., 2013b; Zhang et al., 2014a), among many others (see a long list of references cited in a recent article (Du et al., 2014)). It has also been used in some disciplines of drug development and biomedicine (Zhong and Zhou, 2014) as well as drug-target area (Chou, 2015). Recently, the concept of PseAAC was further extended to represent the feature vectors of DNA and nucleotides (Chen et al., 2013; Chen et al., 2014c; Guo et al., 2014; Liu et al., 2015a; Qiu et al., 2014a), as well as other biological samples (see, e.g., (Jiang et al., 2013)). Because it has been widely and increasingly used, recently three types of powerful open access soft-ware, called 'PseAAC-Builder' (Du et al., 2012), 'propy' (Cao et al., 2013), and 'PseAAC-General' (Du et al., 2014), were established: the former two are for generating various modes of Chou's special PseAAC; while the 3rd one for those of Chou's general PseAAC.

According to (Chou, 2011), PseAAC can be generally formulated as

$$\mathbf{P} = \left[\begin{array}{cccccc} \psi_1 & \psi_2 & \cdots & \psi_u & \cdots & \psi_\Omega \end{array} \right]^{\mathbf{T}} \tag{3}$$

where $\mathbf{T}$ is the transpose operator, while Ω an integer to reflect the vector's dimension. The value of Ω as well as the components ψ_u $(u = 1, 2, \cdots, \Omega)$ in **Eq.3** will depend on how to extract the desired information from a protein sequence. Below, we are to describe how to extract the useful information from the aforementioned benchmark datasets to define a pair of proteins via **Eq.3**.

The wavelet transform (Mallat, 1989) is a very effective approach for using **Eq.3** to formulate a biological sequence, as demonstrated by a series of recent studies such as: (1) using wavelets to formulate PseAAC (Chou, 2001a; Chou, 2005b) for predicting membrane protein types (Liu et al., 2005), predicting protein structural classes (Chen et al., 2012a; Li

et al., 2009), predicting enzyme family classes (Qiu et al., 2010), predicting protein classification (Nanni et al., 2012a), predicting protein quaternary structural attributes (Sun et al., 2012), predicting types of homo-oligomers (Qiu et al., 2011a), as well as predicting G-protein-coupled receptor classes (Qiu et al., 2009), and (2) using wavelets to formulate PseKNC (pseudo-trinucleotide composition) for predicting promoters (Zhou et al., 2013). As is well known, in molecular and cellular biology many remarkable functions in proteins and DNA can be revealed through the low-frequency (or Terahertz frequency) collective motion (Chou, 1988; Chou, 1989b; Chou and Mao, 1988; Gordon, 2008). In view of this, it would be particularly intriguing to define the components of **Eq.3** with the wavelets transform approach because it may help to capture the features important for studying PPI.

Below, we use the wavelets transform to define each of the components in **Eq.3** via the amino acid's physicochemical properties.

1. Physicochemical Properties

Given a protein sample with L residues as expressed by

$$\mathbf{P} = \mathrm{R}_1\mathrm{R}_2\mathrm{R}_3\mathrm{R}_4\mathrm{R}_5\mathrm{R}_6\mathrm{R}_7\cdots\mathrm{R}_L \tag{4}$$

where R_1 represents the 1st amino acid residue of the protein $\mathbf{P}$, R_2 the 2nd residue, and so forth. Different types of amino acid in **Eq.4** may have different physicochemical properties. In this study, we considered the following seven physicochemical properties: (1) hydrophobicity (Tanford, 1962) or $\Phi^{(1)}$; (2) hydrophicility (Hopp and Woods, 1981) or $\Phi^{(2)}$; (3) side-chain volume (Krigbaum and Komoriya, 1979) or $\Phi^{(3)}$; (4) polarity (Grantham, 1974) or $\Phi^{(4)}$; (5) polarizability (Charton and Charton, 1982) or $\Phi^{(5)}$; (6) solvent-accessible surface area (SASA) (Rose et al., 1985) or $\Phi^{(6)}$; and (7) side-chain net charge index (NCI) (Zhou et al., 2006) or $\Phi^{(7)}$. Their numerical values are given in **Table 1**. Thus, the protein $\mathbf{P}$ of **Eq.4** can be encoded into seven different numerical series, as formulated by

$$\mathbf{P} = \begin{cases} \Phi_1^{(1)}\Phi_2^{(1)}\Phi_3^{(1)}\Phi_4^{(1)}\Phi_5^{(1)}\Phi_6^{(1)}\Phi_7^{(1)}\cdots\Phi_L^{(1)} \\ \Phi_1^{(2)}\Phi_2^{(2)}\Phi_3^{(2)}\Phi_4^{(2)}\Phi_5^{(2)}\Phi_6^{(2)}\Phi_7^{(2)}\cdots\Phi_L^{(2)} \\ \Phi_1^{(3)}\Phi_2^{(3)}\Phi_3^{(3)}\Phi_4^{(3)}\Phi_5^{(3)}\Phi_6^{(3)}\Phi_7^{(3)}\cdots\Phi_L^{(3)} \\ \Phi_1^{(4)}\Phi_2^{(4)}\Phi_3^{(4)}\Phi_4^{(4)}\Phi_5^{(4)}\Phi_6^{(4)}\Phi_7^{(4)}\cdots\Phi_L^{(4)} \\ \Phi_1^{(5)}\Phi_2^{(5)}\Phi_3^{(5)}\Phi_4^{(5)}\Phi_5^{(5)}\Phi_6^{(5)}\Phi_7^{(5)}\cdots\Phi_L^{(5)} \\ \Phi_1^{(6)}\Phi_2^{(6)}\Phi_3^{(6)}\Phi_4^{(6)}\Phi_5^{(6)}\Phi_6^{(6)}\Phi_7^{(6)}\cdots\Phi_L^{(6)} \\ \Phi_1^{(7)}\Phi_2^{(7)}\Phi_3^{(7)}\Phi_4^{(7)}\Phi_5^{(7)}\Phi_6^{(7)}\Phi_7^{(7)}\cdots\Phi_L^{(7)} \end{cases} \tag{5}$$

where $\Phi_1^{(1)}$ is the hydrophobicity value of R_1 in **Eq.4**, $\Phi_2^{(2)}$ the hydrophilicity value of R_2,

and so forth. Note that before substituting the physicochemical values of **Table 1** into **Eq.5**, they all are subjected to the following standard conversion

$$\Phi_i^{(\xi)} \Leftarrow \frac{\Phi_i^{(\xi)} - \left\langle \Phi_i^{(\xi)} \right\rangle}{\mathrm{SD}\left(\Phi_i^{(\xi)}\right)} \quad (\xi = 1,\ 2,\ \cdots,\ 7;\ \ i = 1,\ 2,\ \cdots,\ L) \tag{6}$$

where the symbol $\langle\ \rangle$ means taking the average for the quantity therein over the 20 amino acid types, and SD means the corresponding standard deviation. The converted values via **Eq.6** will have zero mean value over the 20 amino acid types, and will remain unchanged if they go thru the same standard conversion procedure again.

2. Discrete Wavelet Transform (DWT)

Wavelet Transform (WT) is a multi-resolution analysis tool (Mallat, 1989). It is quite popular for analyzing, de-noising and compressing signals and images. The WT approach can overcome the shortcoming of Fourier analysis, which is based on the functions that are localized in frequency domain but not in time domain. A digital signal can be decomposed into many groups of coefficients in different scales with WT, and these coefficient vectors can exhibit characteristics in time domain and frequency domain.

The DWT approach can transform a discrete time signal to a discrete wavelet representation. When using the DWT on any of the seven numerical series for protein $\mathbf{P}$ (cf. **Eq.5**), we can view it as a discrete time series, with the 1st residue as $t = 1$, 2nd residue as $t = 2$, and so forth. The discrete time series thus obtained is input into one high-pass filter and one low-pass filter. The coefficients thus obtained can be approximately used for the signal's high scale and low frequency components. In practice, such transform will be applied recursively on the low-pass series with the Mallat algorithm (Mallat, 1999) until the desired number of iterations is reached. The block diagram of **Fig.2** illustrates the digital implementation of DWT. In this study, the decomposition level $\lambda = 4$ was selected to represent a protein, which is similar to the treatment of (Qiu et al., 2011b). Accordingly, we can obtain $(4+1) = 5$ sub-bands when the discrete series $\mathbf{P}$ was decomposed by DWT with level $\lambda = 4$ (see **Fig.2**). Each of the five sub-bands has four coefficients: (1) α_j, the maximum of the wavelet coefficients in the j-th sub-band; (2) β_j the mean of the wavelet coefficients in the j-th sub-band; (3) γ_j the minimum of the wavelet coefficients in the j-th sub-band; (4) δ_j the standard deviation of the wavelet coefficients in the j-th sub-band ($j = 1,\ 2,\ \cdots, 5$). Thus, in a way quite similar to the treatment in (Qiu et al., 2014b; Xu et al., 2013a; Xu et al., 2013b), each of the components in **Eq.3** can be formulated as

$$\psi_u = \begin{cases} \alpha_u & \text{if } 1 \le u \le 5 \\ \beta_u & \text{if } 6 \le u \le 10 \\ \gamma_u & \text{if } 11 \le u \le 15 \\ \delta_u & \text{if } 16 \le u \le 20 = \Omega \end{cases} \quad (7)$$

For a protein pair formed by $\mathbf{P}^{k1}$ and $\mathbf{P}^{k2}$, the corresponding PseAAC can be formulated by their orthogonal sum (Chou and Cai, 2006); i.e.,

$$\mathbf{P}^{k1} \oplus \mathbf{P}^{k2} = \left[\psi_1^{k1} \quad \psi_2^{k1} \quad \cdots \quad \psi_{20}^{k1} \quad \psi_1^{k2} \quad \psi_2^{k2} \quad \cdots \quad \psi_{20}^{k2} \right]^{\mathbf{T}} \quad (8)$$

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 a specified protein $\mathbf{P}^{k1}$ or $\mathbf{P}^{k2}$ instead of a general protein $\mathbf{P}$, and the symbol $\oplus$ represents the sign of orthogonal sum (Chou and Cai, 2006).

Note that when in turn using each of the seven different physicochemical features (cf. **Eq.5**), we can generate seven different PseAAC vectors to represent a same protein pair, as formulated by

$$\text{Protein-pair} = \mathbf{P}^{k1} \oplus \mathbf{P}^{k2}(\xi) = \begin{cases} \text{hydrophobicity} & \xi = 1 \\ \text{hydrophicility} & \xi = 2 \\ \text{side-chain volume} & \xi = 3 \\ \text{polarity} & \xi = 4 \\ \text{polarizability} & \xi = 5 \\ \text{solvent-accessible surface} & \xi = 6 \\ \text{side-chain net charge} & \xi = 7 \end{cases} \quad (9)$$

II.3. Random Forest and Ensemble Classifier

The random forests (RF) algorithm is a powerful algorithm and has been used in many areas of computational biology (see, e.g. (Kandaswamy et al., 2011; Lin et al., 2011; Pugalenthi et al., 2012)). The detailed procedures and formulation of RF have been very clearly described in (Breiman, 2001), and hence there is no need to repeat here.

As shown in **Eq.9**, a protein pair can be formulated with seven different PseAAC forms, each of which can be used to train the RF predictor. Accordingly, we have a total of seven individual predictors for identifying PPIs, as formulated by

$$\text{PPI individual predictor} = \mathbb{RF}(\xi) \qquad (\xi=1, 2, \cdots, 7) \quad (10)$$

where $\mathbb{RF}(\xi)$ represents the RF predictor based on the the ξ-th physicochemical property (cf. **Eqs.5, 6, 9**).

Now, the problem is how to combine the results from the seven individual predictors to

maximize the prediction quality. As indicated by a series previous studies, using the ensemble classifier formed by fusing many individual classifiers can remarkably enhance the success rates in predicting protein subcellular localization (Chou and Shen, 2006b; Chou and Shen, 2007b) and protein quaternary structural attribute (Shen and Chou, 2009b). Encouraged by the previous investigators' studies, here we are also to develop an ensemble classifier by fusing the seven individual predictors $\mathbb{RF}(\xi)$ (ξ=1, 2, ⋯, 7) through a voting system, as formulated by

$$\mathbb{RF}^{\mathrm{E}} = \mathbb{RF}(1)\forall\mathbb{RF}(2)\forall\cdots\forall\mathbb{RF}(7) = \forall_{\xi=1}^{7}\mathbb{RF}(\xi) \tag{11}$$

where $\mathbb{RF}^{\mathrm{E}}$ represents the ensemble classifier, and the symbol $\forall$ denotes 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 (Chou and Shen, 2007a), 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.3** to illustrate how the seven individual RF predictors are fused into the ensemble classifier.

The final predictor thus obtained is called "**iPPI-Esml**", where "i" stands for "identify", "PPI" for "protein-protein interaction", and "Esml" for "ensemble learning".

II.4. Evaluation Metrics and Validation Method

For identifying whether the two counterparts in a pair of proteins are interacting with each other, four metrics 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 (see, e.g., (Chen et al., 2007)). Unfortunately, the conventional formulations for the four metrics are not quite intuitive for most experimental scientists, particularly the one for MCC. Interestingly, by using the symbols and derivation as used in (Chou, 2001b) for studying signal peptides, the aforementioned four metrics can be formulated by a set of equations given below (Chen et al., 2013; Lin et al., 2014; Qiu et al., 2014a)

$$\begin{cases} \mathrm{Sn} = 1 - \dfrac{N_{-}^{+}}{N^{+}} & 0 \le \mathrm{Sn} \le 1 \\ \mathrm{Sp} = 1 - \dfrac{N_{+}^{-}}{N^{-}} & 0 \le \mathrm{Sp} \le 1 \\ \mathrm{Acc} = \Lambda = 1 - \dfrac{N_{-}^{+} + N_{+}^{-}}{N^{+} + N^{-}} & 0 \le \mathrm{Acc} \le 1 \\ \mathrm{MCC} = \dfrac{1 - \left(\dfrac{N_{-}^{+}}{N^{+}} + \dfrac{N_{+}^{-}}{N^{-}}\right)}{\sqrt{\left(1 + \dfrac{N_{+}^{-} - N_{-}^{+}}{N^{+}}\right)\left(1 + \dfrac{N_{-}^{+} - N_{+}^{-}}{N^{-}}\right)}} & -1 \le \mathrm{MCC} \le 1 \end{cases} \tag{12}$$

where N^{+} represents the total number of interactive protein pairs investigated whereas

N_-^+ the number of true interactive pairs incorrectly predicted as the non-interactive pairs; N^- the total number of the non-interactive protein pairs investigated whereas N_+^- the number of non-interactive protein pairs incorrectly predicted as the interactive pairs.

Now with **Eq.12** at hands, it is crystal clear to see the following. When $N_-^+ = 0$ meaning none of the interactive protein pairs is incorrectly predicted to be a non-interactive pairs, we have the sensitivity $\mathrm{Sn} = 1$. When $N_-^+ = N^+$ meaning that all the interactive protein pairs are incorrectly predicted to be non-interactive protein pairs, we have the sensitivity $\mathrm{Sn} = 0$. Likewise, when $N_+^- = 0$ meaning none of the non-interactive protein pairs was incorrectly predicted to be the interactive protein pairs, we have the specificity $\mathrm{Sp} = 1$; whereas $N_+^- = N^-$ meaning that all the non-interactive protein pairs were incorrectly predicted as interactive pairs, we have the specificity $\mathrm{Sp} = 0$. When $N_-^+ = N_+^- = 0$ meaning that none of interactive protein pairs in the positive dataset and none of the non-interactive protein pairs in the negative dataset was incorrectly predicted, we have the overall accuracy $\mathrm{Acc} = 1$ and $\mathrm{MCC} = 1$; when $N_-^+ = N^+$ and $N_+^- = N^-$ meaning that all the interactive protein pairs in the positive dataset and all the non- interactive protein pairs in the negative dataset were incorrectly predicted, we have the overall accuracy $\mathrm{Acc} = 0$ and $\mathrm{MCC} = -1$; whereas when $N_-^+ = N^+ / 2$ and $N_+^- = N^- / 2$ we have $\mathrm{Acc} = 0.5$ and $\mathrm{MCC} = 0$ meaning no better than random prediction. As we can see from the above discussion based on **Eq.12**, the meanings of sensitivity, specificity, overall accuracy, and Mathew's correlation coefficient have become much more intuitive and easier-to-understand.

It should be pointed out, however, the set of metrics as defined in **Eq.12** is valid only for the single-label systems. For the multi-label systems whose emergence has become more frequent in system biology (Chou et al., 2012; Lin et al., 2013; Xiao et al., 2011) and system medicine (Chen et al., 2012b; Xiao et al., 2013b), a completely different set of metrics as defined in (Chou, 2013) is needed.

With the evaluation metrics available, the next thing is what validation method should be used to generate the metrics values.

In statistical prediction, the following three cross-validation methods are often used to derive the metrics values for predictor: independent dataset test, subsampling (or K-fold cross-validation) test, and jackknife test (Chou and Zhang, 1995). Of the three methods, however, the jackknife test is deemed the least arbitrary that can always yield a unique outcome for a given benchmark dataset as elucidated in (Chou, 2011) and demonstrated by Eqs.28-32 therein. Accordingly, the jackknife test has been widely recognized and increasingly used by investigators to examine the quality of various predictors (see, e.g., (Chou and Elrod, 2002; Chou and Cai, 2003; Hajisharifi et al., 2014; Mohabatkar et al., 2013; Mondal and Pai, 2014; Nanni et al., 2014; Shen et al., 2007a; Zhou, 1998; Zhou and Assa-Munt, 2001; Zhou and Doctor, 2003)). However, to reduce the computational time, in this study we adopted the 5-fold cross-validation and 10-fold cross validations, as done by most investigators with random forests algorithm as the prediction engine.

II.4. Web-Server and User Guide

To enhance the value of its practical applications, a web-server for iPPI-Esml has been established at http://www.jci-bioinfo.cn/iPPI-Esml. Furthermore, to maximize the convenience for most experimental scientists, a step-to-step guide or protocol is provided below.

Step 1. Opening the web-server at http://www.jci-bioinfo.cn/iPPI-Esml, you will see the top page of **iPPI-Emsl** on your computer screen, as shown in **Fig.4**. Click on the Read Me button to see a brief introduction about the PPI predictor.

Step 2. Either type or copy/paste the query protein sequences into the input box at the center of **Fig.4**. The input sequence should be in the FASTA format. For the examples of sequences in FASTA format, click the Example button right above the input box.
Step 3. Click on the Submit button to see the predicted result. For example, if you use the query protein sequences in the Example window as the input, you will see the following shown on the screen of your computer: (1) Proteins example-1 and 2 belong to non-interacting pair because their voting score for interaction is $3/7 \approx 0.43$, smaller than $4/7 \approx 0.57$. (2) Proteins example-1 and 3 belong to non-interacting pair because their voting score for interaction is $3/7 \approx 0.43$, smaller than $4/7 \approx 0.57$. (3) Proteins example-2 and 3 belong to interacting pair because their voting score for interaction is $5/7 \approx 0.71 \geq 4/7 \approx 0.57$. All these results are fully consistent with the experimental observations.

Step 4. As shown on the lower panel of **Fig.4**, you may also choose the batch prediction by entering your e-mail address and your desired batch input file (in FASTA format) via the ''Browse'' button. To see the sample of batch input file, click on the button Batch-example.

Step 5. Click on the Citation button to find the relevant papers that document the detailed development and algorithm of **iPPI-Esml**.

Step 6. Click the Supporting Information button to download the benchmark dataset used to train and test the current PPI predictor.

III. RESULTS AND DISCUSSION

The proposed predictor was first tested by the benchmark dataset $\mathbb{S}_{\text{S.C.}}$ in **Eq.1** from *S.cerevisiae*, which contains 17,505 interactive protein pairs and 33,147 non-interactive protein pairs (cf. Online Supporting Information S1). The benchmark dataset was randomly separated into a training dataset $\mathbb{S}_{\text{S.C.}}(\text{train})$ and a testing dataset $\mathbb{S}_{\text{S.C.}}(\text{test})$; i.e.,

$$\mathbb{S}_{\text{S.C.}} = \mathbb{S}_{\text{S.C.}}(\text{train}) \bigcup \mathbb{S}_{\text{S.C.}}(\text{test}) \tag{13}$$

where $\mathbb{S}_{\text{S.C.}}(\text{train})$ contains 5,943 interactive pairs and 5,943 non-interactive pairs, while $\mathbb{S}_{S.C.}(\text{test})$ contains 11,562 interactive pairs and 27,204 non-interactive pairs.

Listed in **Table 2** are the values of the four metrics (cf. **Eq.12**) obtained by **iPPI-Emsl**

via the 5-fold cross-validation on $\mathbb{S}_{S.C.}(\text{train})$. For facilitating comparison, listed in that table are also the corresponding rates obtained by the method proposed by Guo et al. (Guo et al., 2008b).

Listed in **Table 3** are the corresponding results on the (11,562+27,204)=38,766 samples in the independent testing dataset $\mathbb{S}_{\text{S.C.}}(\text{test})$ but trained with (5,943+5943)=11,886 samples in the training dataset $\mathbb{S}_{\text{S.C.}}(\text{train})$.

It can be clearly seen from **Tables 2** and **3**, the new predictor **iPPI-Emsl** remarkably outperformed the Guo et al.' method (Guo et al., 2008b) via both the 5-fold cross-validation and independent dataset tests, indicating the proposed predictor is indeed a quite powerful one.

As mentioned in Section II.1, many state-of-the art prediction methods in this area have used the benchmark dataset $\mathbb{S}_{\text{H.P.}}$ (cf. **Eq.2**) constructed by Martin et al. (Martin et al., 2005) from the cell of *Helicobacter Pylori* to examine their success rates. Below, we are also to use the same benchmark dataset to examine the proposed iPPI-Emsl predictor.

The results obtained by iPPI-Emsl on the benchmark dataset $\mathbb{S}_{\text{H.P.}}$ (cf. Online Supporting Information S2 and Online Supporting Information S3) via the 10-fold cross-validation test are given in **Table 4**, where, for facilitating comparison, the rates obtained by the other methods using exactly the same benchmark dataset and exactly the same cross-validation approach are also given. As we can see from the table, the new method proposed in this paper remarkably outperformed all the other existing methods, once again demonstrating that **iPPI-Emsl** is really a very promising predictor for identifying protein-protein interactions. Particularly, as clearly sown in **Table 4**, in contrast to all the other six existing prediction methods without any web-server provided, the current proposed predictor does provide a use-friendly web-server that is no doubt very useful for the majority of experimental scientists in this or related areas.

Why could the proposed method be so powerful? This is because many key features, which are deeply hidden in complicated protein sequences, can be extracted via the wavelets transform approach. Just like in dealing with the extremely complicated internal motions of proteins, it is the key to grasp the low-frequency collective motion (Chou, 1983; Chou, 1984; Chou and Chen, 1977; Chou et al., 1981; Gordon, 2008; Madkan et al., 2009; Sobell et al., 1983; Zhou, 1989) for in-depth understanding or revealing the dynamic mechanisms of their various important biological functions (Chou, 1988), such as cooperative effects (Chou, 1989b), allosteric transition (Chou, 1987; Schnell and Chou, 2008), assembly of microtubules (Chou et al., 1994), and switch between active and inactive states (Wang and Chou, 2009).

IV. CONCLUSION

In the new PPI predictor, a protein pair is formulated by a general form of PseAAC

whose components are defined via the following procedures: (1) a protein sequence is converted into a numerical series via the physicochemical properties of amino acids; (2) the numerical series is subsequently converted into a 20-D (dimensional) feature vector by means of the DWT technique; (3) the protein pair sample is an orthogonal sum of the two 20-D vectors generated from its two counterparts respectively.

The operation engine to run the PPI prediction is an ensemble classifier formed via a voting system to fuse seven different random forest classifiers based on seven different physicochemical properties, respectively.

Rigorous cross-validations have indicted that the new predictor established with the above procedures is very powerful and promising. It is anticipated that **iPPI-Emsl** will become a very useful high throughput tool for predicting protein-protein interactions in cells, stimulating a series of interesting follow-up researches in this and related areas.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Table 1. The original values of the seven physicochemical properties $\Phi^{(\xi)}$ $(\xi = 1, 2, \cdots, 7)$ for the 20 native amino acids (cf. **Eq.5**).

Amino acid code	$\Phi^{(1)}$	$\Phi^{(2)}$	$\Phi^{(3)}$	$\Phi^{(4)}$	$\Phi^{(5)}$	$\Phi^{(6)}$	$\Phi^{(7)}$
A	0.620	-0.500	27.500	8.100	0.046	1.181	7.187×10^{-3}
C	0.290	-1.000	44.600	5.500	0.128	1.461	-3.661×10^{-2}
D	-0.900	3.000	40.000	13.000	0.105	1.587	-2.382×10^{-2}
E	-0.740	3.000	62.000	12.300	0.151	1.862	6.802×10^{-3}
F	1.190	-2.500	115.500	5.200	0.290	2.228	3.755×10^{-2}
G	0.480	0.000	0.000	9.000	0.000	0.881	1.791×10^{-1}
H	-0.400	-0.500	79.000	10.400	0.230	2.025	-1.069×10^{-2}
I	1.380	-1.800	93.500	5.200	0.186	1.810	2.163×10^{-2}
K	-1.500	3.000	100.000	11.300	0.219	2.258	1.771×10^{-2}
L	1.060	-1.800	93.500	4.900	0.186	1.931	5.167×10^{-2}
M	0.640	-1.300	94.100	5.700	0.221	2.034	2.683×10^{-3}
N	-0.780	2.000	58.700	11.600	0.134	1.655	5.392×10^{-3}
P	0.120	0.000	41.900	8.000	0.131	1.468	2.395×10^{-1}
Q	-0.850	0.200	80.700	10.500	0.180	1.932	4.921×10^{-2}
R	-2.530	3.000	105.000	10.500	0.291	2.560	4.359×10^{-2}
S	-0.180	0.300	29.300	9.200	0.062	1.298	4.627×10^{-3}
T	-0.050	-0.400	51.300	8.600	0.108	1.525	3.352×10^{-3}
V	1.080	-1.500	71.500	5.900	0.140	1.645	5.700×10^{-2}
W	0.810	-3.400	145.500	5.400	0.409	2.663	3.798×10^{-2}
Y	0.260	-2.300	117.300	6.200	0.298	2.368	2.360×10^{-2}

Table 2. The results obtained by the 5-fold cross-validation on the dataset $\mathbb{S}_{\text{S.C.}}(\text{train})$ (cf. **Eq.13**). See **Eq.12** for the definitions of Acc, MCC, Sn, and Sp.

Method	Acc (%)	MCC	Sn (%)	Sp (%)
This paper [a]	84.39	0.6897	87.03	82.13
Guo et al.[b]	77.96	0.5099	76.84	78.22

[a] The proposed predictor **iPPI-Emsl**.
[b] See ref. (Guo et al., 2008b).

Table 3. The results on the (11,562+27,204)=38,766 samples in $\mathbb{S}_{\text{S.C.}}(\text{test})$ but trained with (5,943+5943)=11,886 samples in $\mathbb{S}_{\text{S.C.}}(\text{train})$

Method	Acc (%)	MCC	Sn (%)	Sp (%)
This paper [a]	86.45	0.6832	75.59	91.53
Guo et al.[b]	78.65	0.5171	64.85	85.00

Table 4. Compared with the other six state-of-art methods via the 10-cross-validation on the *H. Pylori* dataset (Martin et al., 2005).

Method	Acc (%)	MCC	Sn (%)	Sp (%)	Web-server
Bock and Gough [a]	75.80	N/A	69.80	80.20	No
Gao 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-Esml [g]	**90.75**	**0.8151**	90.41	87.50	Yes

[a] Results reported by Bock et al. (Bock and Gough, 2003).
[b] Results reported by Guo et al. (Guo et al., 2008a).
[c] Results reported by Martin et al. (Martin et al., 2005).
[d] Results reported by Nanni (Nanni, 2005).
[e] Results reported by Nanni et al. (Nanni and Lumini, 2006).
[f] Results reported by Xia et al. (Xia et al., 2010b).
[g] Results obtained by the current predictor using the same cross-validation method on the same benchmark dataset as the aforementioned six state-of-art-methods.

FIGURE LEGENDS

Figure 1. A complicated protein-protein interaction network is expressed by the vertex-arc graph, where the vertex is used to represent each of the proteins in the network system while the arc to indicate their relation. If there is an arc between two proteins, they are in interaction with each other; otherwise, they are not. For more about using the graphic approach to deal with complicated biological systems, see (Chou, 1989a; Chou, 2010).

Figure 2. A schematic drawing to illustrate the procedure of multi-level DWT (discrete wavelet transform). See the text for further explanation.

Figure 3. A flowchart to show how an ensemble classifier is formed via a voting system.

Figure 4. A semi-screenshot to show the top-page of the iPPIs-Emsl web-server at http://www.jci-bioinfo.cn/iPPI-Esml.

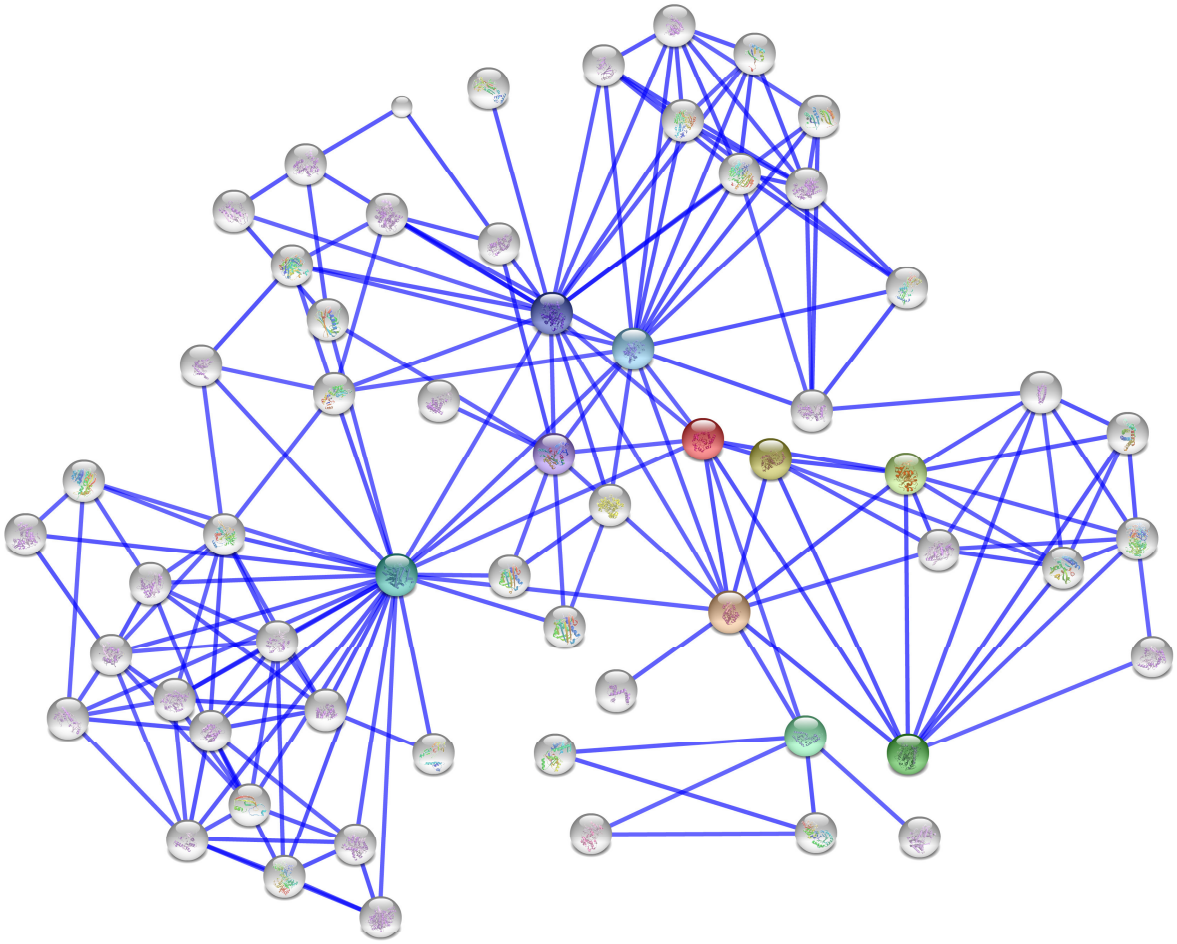

Figure 1

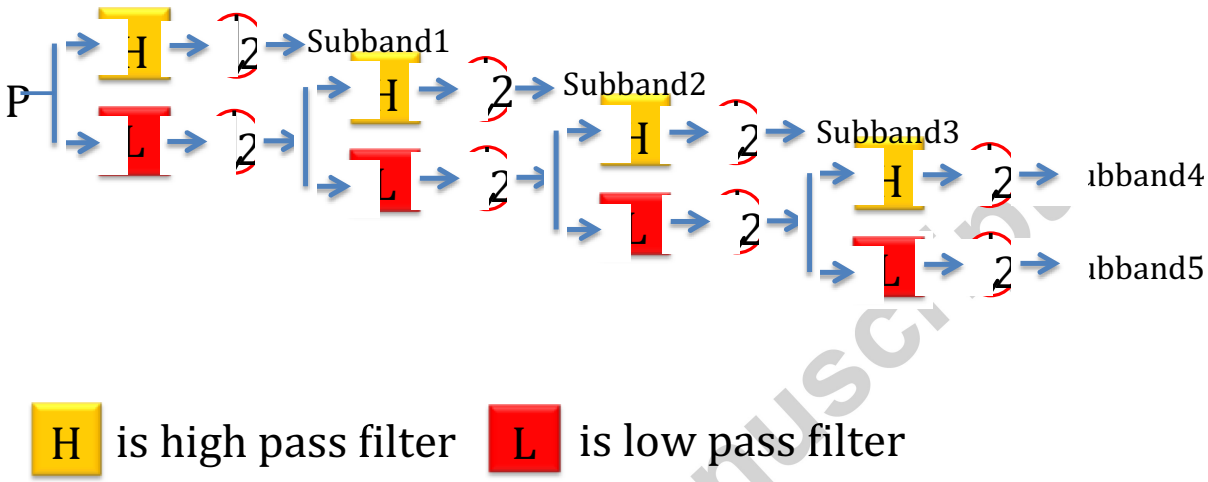

Figure 2

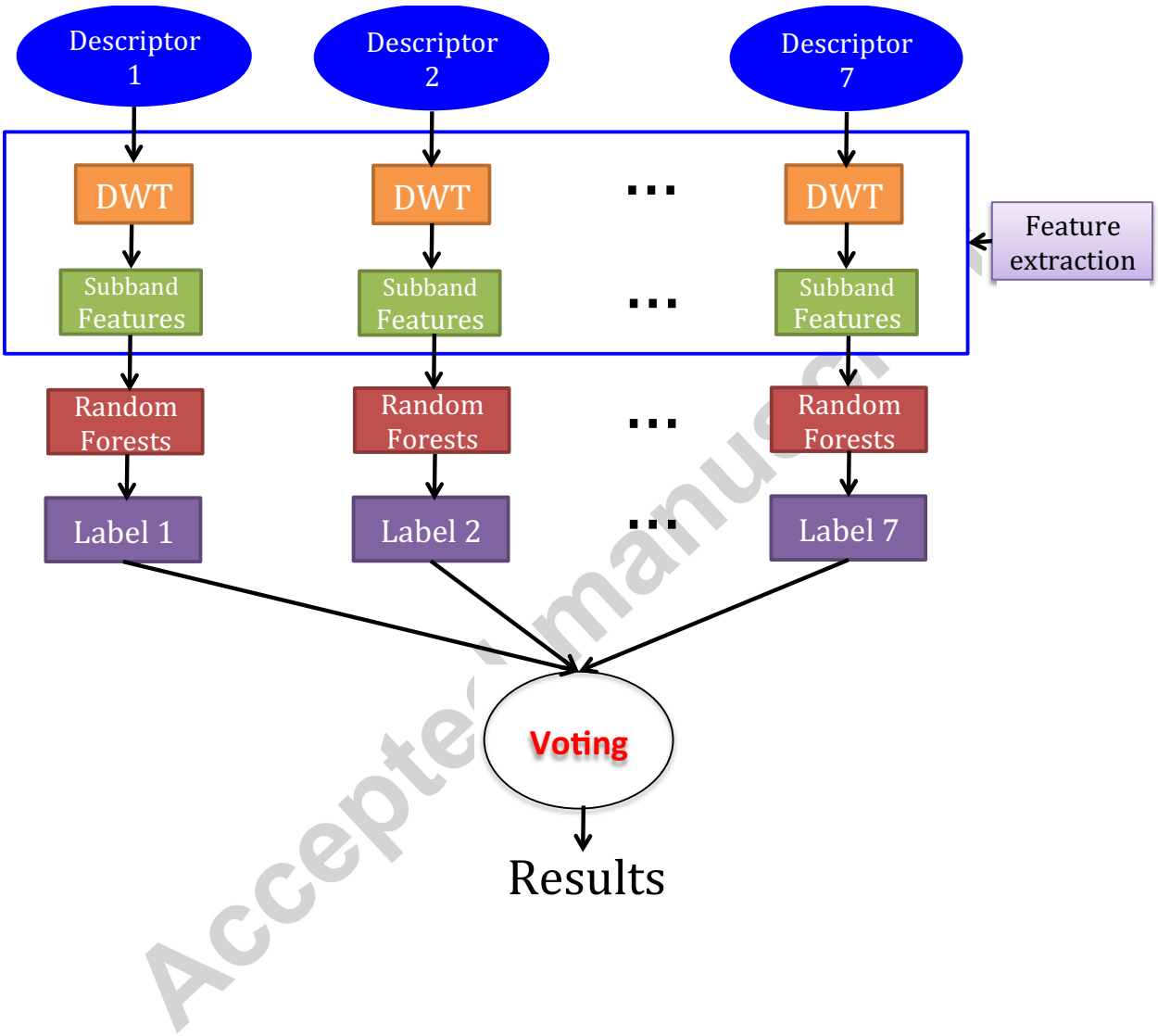

Figure 3

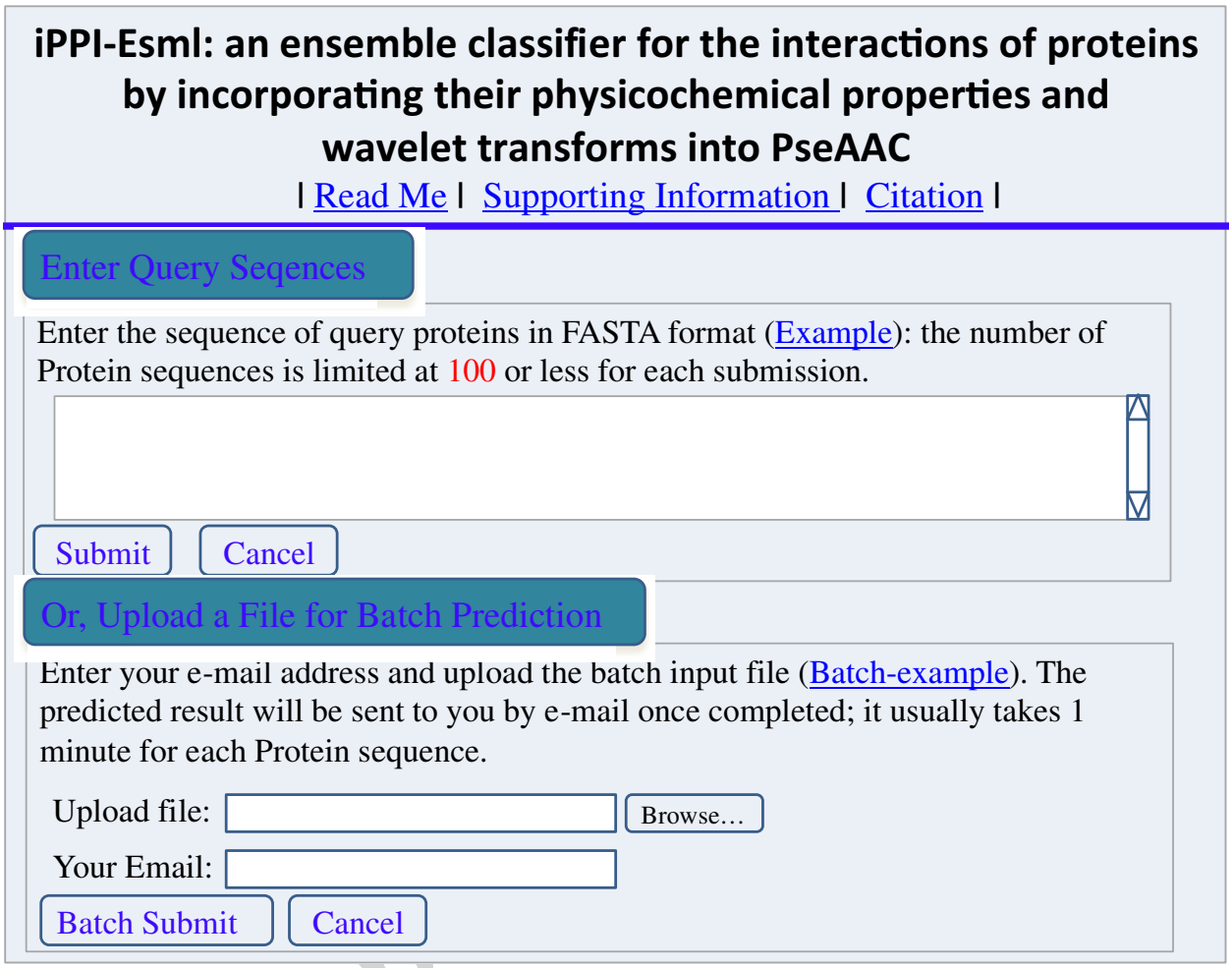

Figure 4

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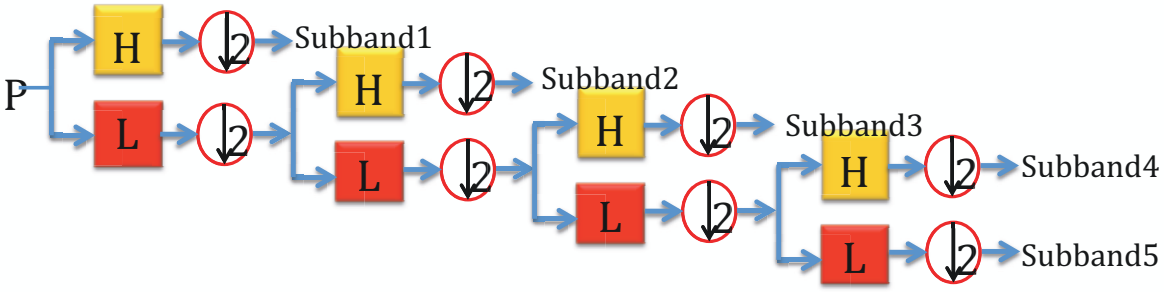
- Protein-protein interactions.
- Discrete wavelet transform approach.
- Ensemble classifier formed by fusing seven individual random forest engines.
- Web-server predictor.



H is high pass filter L is low pass filter

2 is the operator of downsampling with 2