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DELPHI: accurate deep ensemble model for protein interaction sites prediction

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Abstract

Motivation: Proteins usually perform their functions by interacting with other proteins, which is why accurately predicting protein-protein interaction (PPI) binding sites is a fundamental problem. Experimental methods are slow and expensive. Therefore, great efforts are being made towards increasing the performance of computational methods.

Results: We propose DELPHI (DEep Learning Prediction of Highly probable protein Interaction sites), a new sequence-based deep learning suite for PPI sites prediction. DELPHI has an ensemble structure with data augmentation and it employs novel features in addition to existing ones. We comprehensively compare DELPHI to nine state-of-the-art programs on five datasets and show that it is more accurate.

Availability: The trained model, source code for training, predicting, and data processing are freely available at `https://github.com/lucian-ilie/DELPHI`. All datasets used in this study can be downloaded at `http://www.csd.uwo.ca/~ilie/DELPHI/`.

Introduction

Protein-protein interactions (PPI) play a key role in many cellular processes such as signal transduction, transport and metabolism [53]. Proteins interact by forming chemical bonds with other proteins. The bonding amino acid residues are protein-protein interaction sites. Detecting PPI sites helps understand cell regulatory mechanisms, locating drug target, predicting protein functions [8]. Databases like PDB [7] store protein binding sites information deriving from the 3D structure of each protein, but the available protein structures are limited. Experimental methods such as two-hybrid assay and affinity systems are usually time and labour intensive [39]. Computational methods are needed to bridge the gap, and many have been developed [3, 4, 9–11, 15, 18, 20, 22, 23, 25, 28–31, 33, 34, 40, 44, 45, 48, 49, 51, 52, 54]. Out of the above mentioned twenty five computational methods, all but one are machine learning based. Computational methods can be classified into three categories, sequenced based, structure based, and combined. Among them, sequence-based approaches are usually faster and cheaper. They are also more universal because comparing to sequence information, structure information is still limited.

Machine learning methods use feature groups to represent each protein sequence. Widely used features such as position-specific scoring matrix (PSSM), evolutionary

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conservation (ECO), putative relative solvent accessibility (RSA) have been assessed in [55]. High-scoring segment pair (HSP) has been used in previous methods for PPI prediction [27]. One-hot vectors [51, 52] and amino acid embedding [5, 6, 19] have also been empirically explored to represent protein sequences.

The learning structure is crucial to PPI sites classification problems. Previously explored architectures include random forest [45], SVM [45], logistic regression [54], Bayes classifier [33], artificial neural networks [40]. Recently, convolutional neural network (CNN) [51] and recurrent neural network (RNN) [52] have also been applied to solve this problem.

We introduce a new sequence-based PPI sites prediction method, DELPHI (DEep Learning Prediction of Highly probable protein Interaction sites), that combines a CNN and a RNN structure. It uses eleven feature groups to represent protein sequences including two novel features, high-scoring segment pair (HSP) and a reduced 3-mer amino acid embedding (ProtVec1D).

We have comprehensively compared DELPHI with nine state-of-the-art programs on five datasets. DELPHI provides the best overall predictions in all metrics. The contributions of the DELPHI study are as follows. First, a novel fine tuned ensemble model combing CNN and RNN is constructed. Second, a many-to-one structure, that not only serves as a data augmentation technique but also improves the prediction performance, is applied. Third, a data processing and feature construction suite, including new features, is provided, aiming to alleviating the difficulty of tedious feature computation by the users.

Methods

Datasets

Training and validation datasets

A large, high quality dataset was provided in [55]. In this dataset, Uniprot sequences are annotated with Protein, DNA, RNA, and small ligands binding information at the residue level. We further processed this dataset as follows. First, we kept only the sequences with protein-protein binding information to focus on protein-protein binding. Then we removed any sequences from training dataset sharing more than 40% similarities, as measured by CD-hit [17, 26], with any sequences in testing datasets. It is well acknowledged that similar sequences between training and testing datasets negatively affect the generalization of the evaluated performance of a machine learning model. Also, proteins with higher levels of similarity can be accurately predicted by the alignment-based methods [53]. Finally, we ran CD-hit again on the rest of the training protein sequences so no sequences shared more than 40% similarities among training data. This ensures the training data is as diverse as possible. A dataset of 11,063 protein sequences was constructed. From it, we randomly pick 87.5% (9,681) as the training dataset and 12.5% (1,382) as the validation dataset.

Testing datasets

Five datasets are used in the comparative assessment. Four of them are publicly available dataset from previous studies: Dset_186 [33], Dset_72 [21], Dset_164 [13], and Dset_448 [54]. The first three have been widely used and explored by previous studies while Dset_448 is more recent. The raw data of Dset_448 was from the BioLip database [50] where binding sites are defined if the distance between an atom of a residues and an atom of a given protein partner ¡0.5 å plus the sum of the Van der Waal's radii of the two atoms. The raw data was further processed by the author of [54],

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Table 1. The datasets used for training, validation, and the comparative testing. The first column are the dataset names. The second column contains the number of proteins in each dataset. The third, fourth, and fifth columns represent the total number of residue, the number of binding, and the number of non-binding residues in each dataset. The last column represents the percentage of the binding residues out of total.

Dataset	Proteins	Residues			% binding
		total	binding	non-binding	out of total
Dset_448	448	116,500	15,810	100,690	13.57%
Dset_355	355	95,940	11,467	84,473	11.95%
Dset_186	186	36,219	5,517	30,702	15.23%
Dset_72	72	18,140	1,923	16,217	10.60%
Dset_164	164	33,681	6,096	27,585	18.10%
Training + validation	11,063	4,701,678	472,864	4,228,814	10.06%

removing protein fragments, mapping BioLip sequences to UniProt sequences, clustering so that no similarities above 25% is shared within the dataset. The number of sequences, binding, non-binding, and the ratio of binding residues are shown in Table 1. To ensure a fair comparison, we removed 93 proteins in Dset_448 that share more than 40% similarities with DLPred training data and formed a reduced testing dataset of 355 proteins (Dset_355).

Input features

DELPHI uses 11 features groups, shown in Table 2, including high-scoring segment pair (HSP), a variation of 3-mer amino acid embedding (ProtVec1D), position-specific scoring matrix (PSSM), evolutionary conservation (ECO), putative relative solvent accessibility (RSA), relative amino acid propensity (RAA), putative protein-binding disorder, hydropathy index, physicochemical characteristics, physical properties, and PKx. Each input is represented by a 38 dimensional feature vector profile. To the best of our knowledge, this study is the first time that HSP and ProtVec1D are being used in binding sites classification problems. The computation of each of these two new features is described next. High-scoring segment pair (HSP): An HSP is a pair of similar

Table 2. Feature groups used by DELPHI. The first column indicates the name of each feature. The second column describes the program used to obtain the feature. "Load" means the value for a specific amino acid is fixed, and it is loaded in DELPHI program. "Compute" means DELPHI further computes the feature. The last column shows the dimension of each feature group.

Feature	Program	Dimension
High-scoring segment pair (HSP)	SPRINT and compute	1
3-mer amino acid embedding (Pro2Vec1D)	Load and compute	1
Position-specific scoring matrix (PSSM)	Psi-Blast	20
Evolutionary conservation (ECO)	Hhblits	1
Putative relative solvent accessibility (RSA)	ASAquick	1
Relative amino acid propensity (RAA)	Load	1
Putative protein-binding disorder	ANCHOR	1
Hydropathy index	Load	1
Physicochemical characteristics	Load	3
Physical properties	Load	7
PKx	Load	1

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sub-sequences between two proteins. The similarities between two sub-sequence of the same length are measured by scoring matrices such as PAM and BLOSUM.
SPRINT [27] is used for computing all HSPs as it detects similarities fast and accurately among all proteins in training and testing. After obtaining the HSPs, the score for a residue P_i, $\mathrm{HSP}_{\mathrm{score}}(P_i)$, in a testing protein P is calculated as follows. Assume we have an HSP, (u, v), between P and a training protein Q such that u covers the residue P_i and Q_j, the residue of Q at the same relative position in v as P_i is in u, is a known interacting residue. Then we add the PAM120 score between P_i and Q_j; see (1):

$$\mathrm{HSP}_{\mathrm{score}}(P_i) = \sum_{\text{HSPs covering } P_i} \max(0, PAM120(P_i, Q_j)) \tag{1}$$

The 3-mer amino acid embedding (ProtVec1D): We developed this feature based on ProtVec [6]. ProtVec uses word2vec [32] to construct a one hundred dimensional embedding for each amino acid 3-mer. It is shown in [6] that ProtVec can be applied to problems such as protein family classification, protein visualization, structure prediction, disordered protein identification, and protein-protein interaction prediction. Using the ProtVec embedding in our program slows down significantly the deep learning model, especially during training, and also requires a large amount of memory. Therefore, we replaced the one hundred dimensional vector by one value which is the sum of the one hundred components.

Position-specific scoring matrix (PSSM): PSSM matrices are widely used in protein interaction related problems. It contains the evolutionary conservation of each amino acid position by aligning an input sequence with protein databases. The PSSM matrices are computed using PSI-Blast [2] with the expectation value (E-value) set to 0.001 and the number of iterations set to 3. PSI-Blast performs multiple alignment on each input sequence against the non-redundant database.

Evolutionary conservation (ECO): ECO also contains evolutionary conservation, but in a more light-weighted way. To compute the ECO score, the faster multiple alignment tool HHBlits [38] is run against the non-redundant Uniprot20 database with default parameters. The one dimensional conservation value is computed using the formula described in [55].

Putative relative solvent accessibility (RSA): The solvent accessibility is predicted using ASAquick [16]. The values are obtained in the from rasaq.pred file in each output directory.

Relative amino acid propensity (RAA): The AA propensity for binding is quantified as relative difference in abundance of a given amino acid type between binding residues and the corresponding non-binding residues located on the protein surface. The RAA for each amino acid type is computed in [55] by using the program of [43].

Putative protein-binding disorder: The putative protein-binding disorder is computed using the ANCHOR program [14].

Hydropathy index: Hydrophobicity scales is experimentally determined transfer free energies for each amino acid. It contains energetics information of protein-bilayer interactions [47]. The values are computed in [24].

Physicochemical characteristics: For each protein, this includes three features: the number of atoms, electrostatic charges and potential hydrogen bonds for each amino acid. They are loaded from [52].

Physical properties: We use a 7-dimensional property of each amino acid type. They are a steric parameter (graph-shape index), polarizability, volume (normalized van der Waals volume), hydrophobicity, isoelectric point, helix probability and sheet probability. The pre-computed values are loaded from [52].

PKx: This is the negative of the logarithm of the dissociation constant for any other group in the molecule. The values for each amino acid type is loaded from [52].

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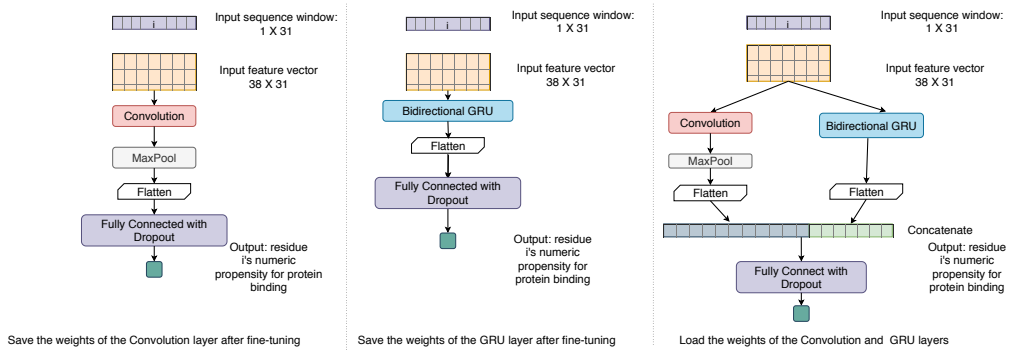

Figure 1. The architecture of DELPHI. Left: the CNN component of the model. Middle: the RNN component of the model. Right: The ensemble model.

After computing all the feature vectors, the values in in each row vector are normalized to a number between 0 to 1 using formula (2) where v is the original feature value, and max and min are the biggest and smallest value observed in the training dataset, resp. This is to ensure each feature group are of the same numeric scale and help the model converge better:

$$v_{\text{norm}} = \frac{v - min}{max - min} \tag{2}$$

Model architecture

DELPHI has an architecture whose model structured is inspired by ensemble learning. The intuition of the design is that different components of the model capture different information, and another deep neural network is trained to only select the most useful ones. As shown in Fig. 2, the first component is a convolutional neural network (CNN), and the second component is a recurrent neural network (RNN). The third part concatenates the outputs of the CNN and the RNN layer and pass it through fully-connected layers.

Another very useful characteristic of the model is its many-to-one structure, meaning that the information of many residues are used to prediction the binding propensity of the centered single residue. As illustrated in Fig. 1, each complete protein sequences are split into sub-sequences of length 31. For each sub-sequence, only the centered amino acid is the prediction target; the rest 30 residues are considered neighbor information that helps the prediction. A sliding window is used to capture each 31-mer. The size 31 is determined experimentally. The beginning and the ending part of the sequence are padded with zeros. The many-to-one structure has two advantages. Firstly, it serves as a data augmentation technique. Deep learning models need large amount of data to train, and comparing to image classifiers, models in proteomics have access to orders of magnitude less data. Using each residue multiple time during the training process helps the model learn better. Secondly, it makes the model more robust. The lengths of protein sequence vary from less than one hundred to several thousand, and most a many-to-many models have a fixed input length of near 500. Any sequences that are shorter or longer than the set value need to be either padded long or cut into pieces. This differs from how the programs is trained where all proteins are picked near the set value, and thus decreases the prediction performance.

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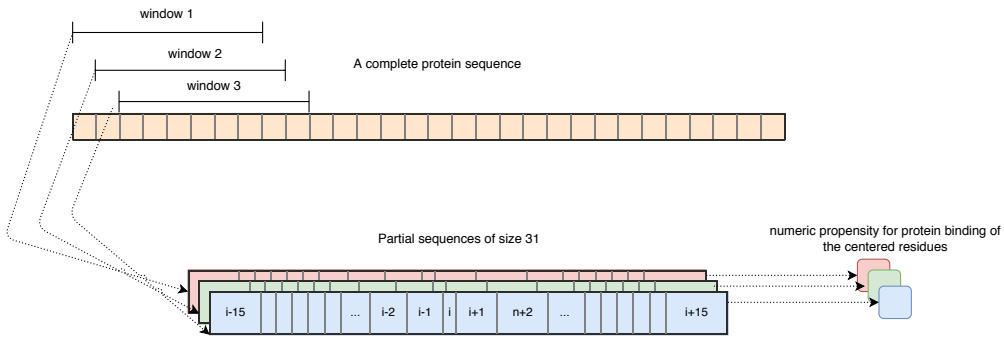

Figure 2. The many-to-one prediction. Sliding windows of size 31, stride 1 are put on top of an input protein sequence. Each time, a sub-sequence of length 31 is extracted. The model predicts the protein-binding propensity of the middle amino acid for each sub-sequence.

Architecture of the CNN network

The CNN model one has a concise structure. For each input sub-sequence of size 31, a 2D feature profile of size 38 by 31 is constructed. The 2D vector is reshaped into 3D and then passed to a convolution 2D layer, followed by a maxpooling layer. The intuition of using the convolution and maxpooling layers is that a 2D protein profile vector can be considered as an image with one channel, and the CNN model captures the combination of several features in a partial image. The results are flattened and then fed into two fully connected layers with dropout for regularization. The last fully connected layer has one unit with activation function sigmoid, so that the output is a single value between 0 to 1. The higher the value, the more confident the CNN model claims that the residue is a protein interaction binding site.

Architecture of the RNN network

Similar to the CNN component, the 2D feature profile of size 38 by 31 is built for each 31-mer. The feature profile is passed to a bidirectional gated recurrent units (GRU) layer with the intention to memories the dependency and relationship among the 31 residues. We set the GRU layer to return a whole sequence as opposed to return a single value. The results are flattened and fed into two fully connected layers with dropout. The output of the RNN network is also a single value between 0 to 1.

Architecture of the ensemble network

The final model combines the core layers of the above mentioned CNN and RNN models and tries to further extract essential information of protein binding. The ensemble network takes a sequence of length 31 as its input. Similar to the CNN and RNN components, a 38 by 31 feature vector is constructed and passed to both a convolution layer and a bidirectional GRU layer. The output of the convolution layer is passed on to a maxpool layer and then flattened. The GRU output is also flattened. Finally the outputs of the two flatten layers are concatenated and passed on to two fully connected layers with dropout. The last fully connected layer has one output unit with a sigmoid

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activation function, so the final output is a single value between 0 to 1, indicating the propensity of protein binding. This is the final output of the entire model.

Fine tuning is used in this ensemble model. The convolution layer in the CNN network and the bidirectional GRU layer in the RNN network are tuned separated using the same training/validation dataset. After achieving the best performance on the CNN and the RNN components, the weights of the convolution and the GRU layer are saved to files. In the ensemble mode, the convolution and the GRU layer load the saved weights from the file and freeze the weights, so that during the process of training, the convolution and the GRU layer stay unchanged. Training and validation data are used again only to train the fully connected layers in the ensemble model.

Implementation

The program is written in Keras [12] with TensorFlow GPU [1] back end. All features are computed from sequence only. We alleviate the burden of feature computation from users by providing all computation programs and a pipeline script. We ease the system configuration process by providing users a pip package list which enables one-command installation.

Classifying protein binding residue is an imbalanced problem. To cope with that, different class weights [42] are assigned to the positive and negative samples. The values are determined by the ratio between binding residues over all residues in the training data.

During training, we shuffle the data before each epoch. Since the sliding window is used to extract the 31-mer, adjacent data entries are very similar; only the first and the last residue differ from the previous and the next data entry. Shuffling the whole training data diversifies the input in each batch, and thus helps the model to converge better.

Parameter tuning

Parameters and hyper-parameters are chosen based on the training dataset while applying early stopping [37] on the validation set. Early stopping halts the training process when a performance drop on the validation set is detected. This is to avoid overfitting the training dataset. We chose all parameters with the purpose to maximize area under the recall-precision curve (AUPRC) on the training data. All testing results are then carried using the already tuned model. All parameters and hyper-parameters used in this model are shown in Table 3.

The DELPHI model takes 2.1 hours to train the CNN component, 0.5 hour to train the RNN component and 1.3 hours to train the ensemble model on our testing cluster.

Results

Competing methods

We have comprehensively compared DELPHI with nine state-of-the-art machine learning based methods. The methods are selected using the following criteria. First, the program is a sequence-based method as sequence information is readily available for most proteins. Second, the program is available in the form of source code or a web server. Lastly, the program takes in any input sequence in fasta format and produces the results on an average length protein within thirty minutes. Following the criteria, DLpred [52], SCRIBER [54], SSWRF [45], SPRINT [41], CRF-PPI [46], LORIS [13], SPRINGS [40], PSIVER [33], and SPPIDER [36] are selected.

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Table 3. Parameters used in DELPHI. Parameters are divided into four groups: CNN, RNN, ensemble model, and hyper-parameters.

Parameter	Value
Epoch in CNN	15
Kernel size in CNN	5
Stride in CNN	1
Padding in CNN	valid
Number of filters in CNN	64
Fully connected unit in CNN	64, 1
Epoch in RNN	5
GRU unit	32
Fully connected unit in RNN	64, 1
Epoch in ensemble	17
Fully connected unit in ensemble	64, 1
Batch size	1024
Dropout rate	0.3
Optimizer	Adam ($\beta_1 = 0.9, \beta_2 = 0.999$)
Patience in early stop	7
Loss function	binary cross entropy
Learning rate	0.002

All tests have been performed on a Linux (Ubuntu 16.04) machine with 24 CPUs (Intel Xeon v4, 3.00GHz), 256GB memory, and a Nvidia Tesla K40c GPU.

Evaluation scheme

Similar to previous studies, we use sensitivity, specificity, precision, accuracy, F1-measure, (F1), Matthews correlation coefficient (MCC), area under the receiver operating characteristic curve (AUROC), and area under the precision-recall (AUPRC) to measure the prediction performance. All programs output a prediction value for each amino acid, and thus the operating characteristic curve (ROC) and the precision-recall (PR) curve can be drawn. AUROC and AUPRC are also computed based on the curves using tools such as [35]. We focus more on AUROC and AUPR because they are threshold independent and convey an overall performance measurement of a program. The rest of the metrics are calculated using a binding threshold which is determined after obtaining the prediction scores from each program. Since each program's output is of different scale, for each program, we pick the threshold such that for a given testing dataset, the number of predicted scores above the threshold is equal to the real number of binding sites in the dataset.

The formulas for calculating the metrics are as follows, where true positives (TP) and true negatives (TN) are the correctly predicted binding sites and non-binding sites, respectively, and false positives (FP) and false negative (FN) are incorrectly predicted binding sites and non-binding sites, respectively.

$$Sensitivity = \frac{TP}{TP + FN} \tag{3}$$

$$Specificity = \frac{TN}{TN + FP} \tag{4}$$

$$Precision = \frac{\mathrm{TP}}{TP + FP} \tag{5}$$

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$$Accuracy = \frac{TP + TN}{TP + FN + TN + FP} \tag{6}$$

$$F1 = 2 \times \frac{Sensitivity \times Precision}{Sensitivity + Precision} \tag{7}$$

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

Comparative assessment of predictive performance

Performance comparison on Dset_448 and Dset_355

We first compare the DELPHI model with eight programs on Dset_448. This dataset is the most recently published and has the largest number of proteins, so we emphasize the importance of this dataset. As shown in Table 4, DELPHI surpasses competitors in all metrics with an improvement of 3% and 12.2% on AUROC and AUPR respectively comparing to the second best program SCRIBER.

Table 4. Performance comparison of nine programs on Dset_448. Programs are sorted in asending order by AUPRC. Darker colours represent better results. The evaluation of the programs marked with * are carried by [54].

Predictor	Sensitivity	Specificity	Precision	Accuracy	F1	MCC	AUROC	AUPRC
Dset_448								
SPPIDER*	0.202	0.870	0.194	0.781	0.198	0.071	0.517	0.159
SPRINT*	0.183	0.873	0.183	0.781	0.183	0.057	0.570	0.167
PSIVER*	0.191	0.874	0.191	0.783	0.191	0.066	0.581	0.170
SPRINGS*	0.229	0.882	0.228	0.796	0.229	0.111	0.625	0.201
LORIS*	0.264	0.887	0.263	0.805	0.263	0.151	0.656	0.228
CRFPPI*	0.268	0.887	0.264	0.805	0.266	0.154	0.681	0.238
SSWRF*	0.288	0.891	0.286	0.811	0.287	0.178	0.687	0.256
SCRIBER	0.334	0.896	0.332	0.821	0.333	0.230	0.715	0.287
DELPHI	0.354	0.899	0.354	0.825	0.354	0.252	0.737	0.322

In order to have a more comprehensive comparison, we compare DELPHI with another recent sequence-based program, DLPred. However, the training data in DLPred has a big overlap with Dset_448, so we removed the highly similar sequence in Dset_448 using the protocol described in section and produced a reduced dataset Dset_355 which is tested in Section .

Performance comparison on Dset_355, Dset_186, Dset_164, and Dset_72

To further compare DELPHI with other programs, we used Dset_355 along with another three previously published datasets: Dset_186, Dset_164, and Dset_72. Since DLPred and SCRIBER are the most recent programs and have been shown to outperform older programs, we compare DELPHI only with SCRIBER and DLPred on the remaining datasets. As shown in Table 5, DELPHI clearly outperforms the competitors in all metrics on Dset_355. SCRIBER are DLPred are very close to each other's performance. DLPred is slightly better than SCRIBER in AUROC, and SCRIBER is slightly higher in AUPRC. As shown in Table 5, The AUROC of DELPHI is the best in all datasets, while the AUPRC is the best in two datasets and is the second best in Dset_164. This is might because (1) the binding residue percentage in Dset_164 is the highest in all

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Table 5. Performance comparison of SCRIBER, DLPred, and DELPHI on Dset_355, Dset_186, Dset_164, and Dset_72. Darker colours represent better results. Each dataset is tested separately using the same metrics. The average metrics of the three datasets is also shown.

Predictor	Sensitivity	Specificity	Precision	Accuracy	F1	MCC	AUROC	AUPRC
Dset_355								
SCRIBER	0.322	0.908	0.322	0.838	0.322	0.230	0.719	0.275
DLPred	0.308	0.906	0.308	0.835	0.308	0.214	0.724	0.272
DELPHI	0.348	0.911	0.348	0.844	0.348	0.259	0.747	0.311
Dset_186								
SCRIBER	0.279	0.870	0.279	0.780	0.279	0.150	0.647	0.246
DLPred	0.320	0.878	0.320	0.793	0.320	0.198	0.694	0.290
DELPHI	0.351	0.884	0.351	0.803	0.351	0.235	0.715	0.327
Dset_164								
SCRIBER	0.327	0.851	0.327	0.756	0.327	0.179	0.657	0.301
DLPred	0.338	0.854	0.338	0.760	0.338	0.192	0.672	0.330
DELPHI	0.332	0.852	0.332	0.758	0.332	0.184	0.679	0.304
Dset_72								
SCRIBER	0.232	0.909	0.232	0.837	0.232	0.141	0.680	0.198
DLPred	0.246	0.901	0.246	0.826	0.246	0.148	0.688	0.215
DELPHI	0.275	0.915	0.275	0.847	0.275	0.189	0.707	0.237
Average								
SCRIBER	0.290	0.885	0.290	0.803	0.290	0.175	0.676	0.255
DLPred	0.303	0.885	0.303	0.803	0.303	0.188	0.695	0.277
DELPHI	0.326	0.891	0.326	0.813	0.326	0.217	0.712	0.294

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datasets as shown in Table 1 and (2) DLpred is trained on a collection of data with higher ratio of binding residue [52], so that it is more suitable for datasets like Dset_164. We present also the average values in Table 5 the average values over Dset_355, Dset_186, Dset_164, and Dset_72 for each specificity value. The performance of DELPHI again surpasses all competing programs.

Software availability

DELPHI is an open source program under the GPLv3 License. The trained model, source code of training, predicting, and data processing is freely available at `https://github.com/lucian-ilie/DELPHI`. All datasets used in this study can be downloaded at `http://www.csd.uwo.ca/~ilie/DELPHI/`.

Conclusion

We have presented a new deep learning model and program DELPHI, for predicting PPI binding sites. We compared DELPHI with nine current state-of-the-art programs on five datasets and demonstrated that DELPHI has a higher prediction performance. There is still plenty room for improvement on this topic as the highest AUC in all test is 0.747. We hope in the future, the model architecture, the usage of the two new features, and the many-to-one structure can be extended to predicting protein biding with other types of molecules, such as DNA, RNA, and ligand.

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