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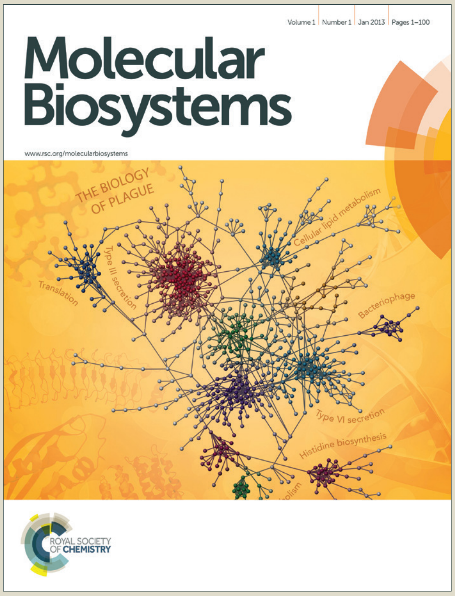

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Protein submitochondria localization from integrated sequence repesentation and SVM-based backward feature extraction

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Abstract

Mitochondrion, a tiny energy factory, plays an important role in various biological processes of most eukaryotic cells. Mitochondrial defection is associated with a series of human diseases. Knowledge of the submitochondrial locations of proteins can help to reveal the biological functions of novel proteins, and understand the mechanisms underlying various biological processes occurring in the mitochondrion. However, experimental methods to determine protein submitochondria locations are costly and time consuming. Thus it is essential to develop a fast and reliable computational method to predict protein submitochondria locations. Here, we proposed a support vector machine (SVM) based approach for predicting protein submitochondria locations. Information from position-specific score matrix (PSSM), Gene Ontology (GO) and protein feature (PROFEAT) was integrated into the principal features of this model. Then a recursive feature selection scheme was employed to select the optimal features. Finally, an SVM module was used to predict protein submitochondria locations based on the optimal features. Through the jackknife cross-validation test, our method achieved an accuracy of 99.37% on benchmark dataset M317, and 100% on the other two datasets, M1105 and T86. These results indicate that our method is ecnomic and effective for acurate prediction of the protein submitochondrial location.

Keywords

Submitochondria location; Position-specific score matrix; Gene Ontology; PROFEAT; Support vector machine-Recursive feature elimination

Introduction

Mitochondria plays an important role in various biological processes [1-3], including programmed cell death, oxidative phosphorylation, ion hemostasis and innate immune activation. A series of human diseases [4-6], such as Parkinson's disease, diabetes mellitus, epilepsy, cardiac ischemia/reperfusion injury, Alzheimer's disease and cancer, are associated with mitochondrial defects. Since the function of proteins are highly correlated with their locations, knowledge of the protein submitochondria location can be very helpful for understanding mechanisms of mitochondrial defects related diseases and developing novel drugs. As biochemical experiments [7] are time-consuming, tedious and costly. With a large number of protein sequences generated in the post-genomic age, it is highly desirable to develop effective computational systems to address this problem. Up to now, there are only few computational methods for identifing protein submitochondria location [7, 8], and their efficiencies are still not satisfactory. Therefore, a novel method for accurate and reliable protein submitochondria location prediction is essential.

As a typical classification task, computational model for protein submitochondria localization consists of the following three components: i) protein feature representation; ii) algorithm selection for classification; iii) optimal feature selection. Formulating the protein sample by an effective mathematical expression is a critical factor to develop a powerful predictor for a protein system [9]. Various methods have been proposed to extract features for protein localization prediction [10-13], which are commonly based on the protein sequence or sequence-related information, such as Terminal signaling peptides, amino acid composition (AAC), pseudo amino acid composition (PseAAC), polypeptide composition, functional domain composition, Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) profile, and amino acid sequence reverse encoding. Compared with traditional monolithic approaches base on a single feature, the methods based on fusing multiple features have been widely used to improve the prediction performance in the protein subcellular prediction. In this study, we attempted to represent the protein sample through the fusion of information obtained from PROFEAT, gene ontology (GO) and PSSM. PROFEAT is a web server for retrieving frequently used sequence-derived features of proteins, such as amino acid composition, Geary autocorrelation, or sequence-order-coupling number[14]. While the GO could provide a dynamic controlled vocabulary of terms for describing gene product characteristics and gene product annotation data from GO Consortium member [15], enhancing the success rate in prediction significantly [16]. We previously applied GO annotation to improve the prediction of multi-location protein subcellular localization [17]. Besides, the position-specific score matrix (PSSM)[18], derived from the PSI-BLAST program, contains the evolutionary information as well as some essential signatures of the protein families. PSSM-based features were often used to detect distant homology, especially in low similarity datasets.

After representing protein sequence as a fixed-length numerical vector, a powerful classification algorithm should be used to operate the prediction. Many machine learning algorithms were developed for protein analysis in the last decade, such as the support vector machine (SVM), artificial neural network, fuzzy K-nearest neighbor (NN), optimized evidence-theoretic (OET)-KNN genetic algorithm and the Markov model. In this study, we used SVM to operate submitochondria localization prediction for its flexibility, high computational efficiency and good generalization in high-dimensional input spaces in many classification tasks [19, 20]. However, the original SVM format lacks the ability to filter out irrelevant, redundant or noise features, which may affect the system performance, including classification accuracy and computational efficiency. Thus, selecting relevant features is an important task in protein submitochondria localization prediction.

Commonly used feature selection techniques can be attributed into three categories: filter, wrapper and embedded methods. Compared to filter and wrapper, Embedded methods could avoid high risk of overfitting and ignorance of feature dependencies by taking feature correlations into account and discretely removing only one feature from the whole feature vectors. Thus it is much more robust to data overfitting than other feature selection approaches [21]. Generally, with the ability to take feature dependencies into account, embeddeds can yield better performance than other methods. Recursive Feature Elimination (SVM-RFE) [21] is one of the most popular embedded methods for SVMs. SVM-RFE conducts feature selection in a sequential backward elimination manner, which starts with the whole features and removes one feature each time. Some previous reports showed that features selected by SVM-RFE yield good classification performance in many applications, such as biomarker selection, gene selection, tissue detection [22] and so on.

In this study, an SVM-based model was developed to improve the prediction of protein submitochondria locations with recursively selecting features from PSI-BLAST profile, physical-chemical properties and protein functional annotations. Before inputted to an SVM classifier to perform the prediction, critical features were selected by SVM-RFE and prediction quality was examined by jackknife tests on three datasets. The results of all prediction performances show that our proposed approach is superior to those methods [8, 23-27] ever reported.

Materials and methods

1. Datasets

In this study, three benchmark datasets [26, 27] were used to evaluate the performance of our method (**Table 1**): M1105 dataset includes 1105 proteins distributed into 3 submitochondria locations. M317 dataset includes 317 proteins classified into 3 submitochondria locations. T86 dataset is an independent test dataset that includes 86 human mitochondria proteins and also classified into 3 locations. None of the proteins in the three datasets has ≥40% sequence identify to any other in the same subset.

2. Feature preparation

To develop a powerful predictor for protein analysis, one of the most important problems is how to formulate a protein sample with an effective mathematical expression or a discrete model that could keep considerable sequence order infotmation. To realize this, the concept of pseudo amino acid composition [28] or Chou's PseAAC [29] was proposed for representing the sample of a protein. Ever since the concept of PseAAC was introducted, it has been widely used in most of the areas of computational proteomics [30, 31]. After the web-server 'PseAAC' [32] was established, three effective open access softwares, i.e., ‘PseAAC-General’ [33], ‘propy’ [34], and ‘PseAAC-Builder’ [35], were also built for the purpose. The first is for generating the general model of PseAAC, while the latter two for various modes of special PseAAC. In this work, we are to use a combination of evolutionary information, GO information and physicochemical/structural features to represent the protein samples via PseAAC.

2.1. Linear predictive coding of the PSI-BLAST profiles

The evolutional information involved in PSSM is highly useful for evaluate relationships in database searches. In this study, PSSM extracted from sequence profiles generated by PSI-BLAST was selected as the feature descriptor. We used the PSI-BLAST tool and NCBI non-redundant (NR) dataset on a local machine for creating PSSM for all proteins. The paremeters *j* and *h* are set to 3 and 0.001, respectively. Every PSSM element was scaled to the range from 0 to 1 using the standard sigmoid function:

$$f(x) = \frac{1}{1+e^{-x}} \qquad (1)$$

where x is the original PSSM value.

Then, the linear predictive coding (LPC) scheme [36], a tool used mostly in audio signal processing and speech processing, was employed to parameterize the optimal signal. LPC is one of the most powerful speech analysis techniques, and provides extremely accurate estimates of speech parameters. The derived coefficients were used as quantitative features replacing signal intensities. Here, we used LPC analysis process to extract p features for each column of PSSM, and a $20\times p$ feature vector was transformed from the PSSM for each protein.

2.2. Gene function annotation features

GO term data were obtained from ftp://ftp.ebi.ac.uk/pub/databases/GO/goa/UNIPROT/ (released on May 7, 2014). All accession numbers in three datasets were searched against the GO database to find the corresponding GO numbers. As the current GO terms did not cover all proteins, BLAST was used to search homologous proteins of protein ***P*** without known GO terms under the expect parameter $E\leq0.001$, and collected proteins with ≥60% pairwise sequence similarity to ***P***. Then, the geometrical center of these homologous GO features was used to represent protein ***P***. Thus, we obtained 1,569, 879 and 423 different GO terms for M1105, M317 and T86, respectively. Finally, a feature vector was created to represent the GO terms for each protein as described in ref [22]. Due to its low sequence similarity and large population size, M1105 was used to optimize the parameters in LIBSVM [37], and implemented to predict the submitochondria location of a query protein.

2.3. Structural and physicochemical features by PROFEAT

PROFEAT was designed for computing commonly-used structural and physicochemical features of proteins and peptides from their primary sequences [14, 38]. These features include amino acid composition, dipeptide composition, Moran autocorrelation, sequence-order-coupling number, Geary autocorrelation, normalized Moreau–Broto autocorrelation and the composition, transition and distribution of various structural and physicochemical properties. Moreover, new feature groups such as pseudo-amino acid composition (PAAC), amphiphilic PAAC (APAAC), total amino acid properties (TAAPs), and atomic-level topological descriptors are added in the new version of PROFEAT. The enhancements facilitate prediction of proteins, peptides, small molecules of different properties and molecular interactions. In this study, for a query protein, the sequence was inputted and all the PROFEAT features were selected. As a result, we got a 1080-dimension vector of PROFEAT feature.

3. Feature extraction by SVM-RFE

Due to the limitation of training data, a small amount of features usually result in a better generalization of machine learning algorithms (Occam's razor) [39]. To select a set of key features for reliable prediction of protein submitochondria locations, an SVM-RFE algorithm has been developed. Firstly, PSSM, PROFEAT and GO features of each protein were merged into a feature vector. All the feature vectors of proteins for each dataset were used to construct a feature matrix, where each row represents a sample and each column represented a feature. Then, training an SVM with a linear kernel, we ran the SVM-RFE algorithm to get a rank list of all features by removing only one feature with the smallest ranking criterion each time. The first item in the rank list was the most relevant to perform protein submitochondria location prediction, and the last item had the least relevant feature. Finally, we were able to select different top *K* features according to the ranking list.

4. The SVM ensemble classifier

Due to excellent generalization capabilities to converge to a single globally optimal solution, SVM is widely used in the bioinformatics applications [40-42], including predictions of protein subcellular location, membrane protein types, protein crystallization, zinc-binding sites and protein-binding RNA nucleotides. Compared to several other methods, SVM has some merits including the robustness against several types of model violations and outliers, the ability to learn well with only a few free parameters, and the computational efficiency [43]. Due to the performance of SVM is decided by the type of kernel function, we used the most popular radial basis function (RBF) kernel [44] for its good performance in different prediction tasks. When training an RBF kernel SVM, we considered the parameter γ and regularization parameter *C*, which could affect the performance of protein submitochondria location prediction. In this study, the two parameters were also optimized based on M1105 dataset by a grid search strategy.

Prediction of protein submitochondria locations is usually formulated as a multi-class classification problem. This requires a multi-class analysis be broken down into a series of binary classifications, following either the *one-against-one* or the *one-against-rest* approach [45]. In this study, the *one-against-one* strategy was employed for its better symmetry than *one-against-rest* strategy. Therefore, 3´ 2/2 = 3 binary classification tasks were constructed for each dataset. However, feature vectors optimized by different datasets showed slight difference (**Fig. 1**). Finally, the SVM module predicted the submitochondria location of a protein using the top features and the optimal combination of the two parameters.

5. Assessment of prediction performance

In statistical prediction, the independent dataset test, subsampling test and jackknife test are three evaluation methods often used to examine a predictor for its prediction accuracy in practical applications [46]. Among them, the jackknife test seems to be the most objective and rigid [47], and thus was adopted in this study. The accuracy, overall accuracy and Matthew's Correlation Coefficient (MCC) [48] were defined by:

$$accuracy(i) = \frac{TP_i}{n_i} \qquad (2)$$

$$overall\,accuracy = \frac{\sum_{i=1}^{M} TP_i}{N} \qquad (3)$$

$$MCC(i) = \frac{TP_i \times TN_i - FP_i \times FN_i}{\sqrt{(TP_i + FP_i)(TP_i + FN_i)(TN_i + FP_i)(TN_i + FN_i)}} \qquad (4)$$

Here, N denotes the total number of proteins, M denotes the class number, n_i is the number of proteins in class i. TP_i, FP_i, TN_i and FN_i denote true positives, false positives, true negatives, and false negatives in class i, respectively. It is instructive to point out that the above equation set is often used in literatures [49-51] for examining the performance quality of a predictor. For an intuitive interpretation about these metrics, particularly for Eq.4, see the aforementioned papers. The set of metrics is efficacious only for the single-label systems. For the multi-label systems which were frequently existent in system biology [52], an absolutely different set of metrics was defined in [53]. A flowchart was provided in **Fig. 2** to illustrate the prediction process of this method.

Results and discussion

1. Parameter selection

The parameter *γ* of RBF kernel and regularization parameter *C* in LIBSVM should be determined to calculate the prediction accuracy. In this study, we ultilized a grid search strategy in M1105 dataset to select them via computing the best dimension *Dim* of protein top feature vector. Firstly, we built up an initial feature vector, which was integrated by PSSM, PROFEAT and GO features. Secondly, according to their importance, a ranking list of all the features was returned based on SVM-RFE. According to the ranking list, we calculated the prediction accuracies for top *N* features, where $N = 10 \times 2^{n-1}\,(n = 1, 2, 3, \cdots, 8)$. We found that the accuracy at top80 ($n = 4$) reached 100% for M1105 dataset (**Fig. 3**). Finally, top80 features and the corresponding parameters ($C = 512$, $\gamma = 1.221 \times 10^{-4}$, and $Dim = 80$) were chosen as the optimal parameter group to calculate the accuracies for all three datasets.

As shown in **Fig. 1**, GO features consistently make up the majority of top80 features in each dataset, followed by PROFEAT and PSSM in turn. More than half of the top80 selected features were GO features for all three datasets. For instance, the number was up to 54 for M1105. These results indicated that the subcellular localization of a protein could be characterized by GO features.

2. Comparison with other methods

To assess the performance of our predictor, we compared our method with several other previous methods on the three benchmark datasets with a jackknife test. Our method attained the best overall accuracy of 99.37%, which was higher than those achieved using methods [8, 23-26] listed in **Table 2** (from 14.17% to 4.42%). Moreover, in terms of the accuracy and MCC of all the three sites, our method also ranked the first. We noticed that the second best method listed in **Table 2** also used the combined features and SVM algorithm, proving that the merged features and SVM algorithm were powerful for inferring the submitochondria location. We introduced dataset M1105 to further validate our predicting performance. We compared the results of our method and the method constructed by Fan [26]. As can be seen from **Table 3**, our method achieved an overall accuracy of 100.00%, outperformed the latter in terms of the overall accuracy, as well as the accuracy and MCC of all the location sites. Of note, the accuracy of outer membrane in this method was 13.1% higher than that of the latter, suggesting that our method worked well on predicting submitochondria location. In fact, there are several GO terms describing submitochondrial locations. It could be a possible reason for our good performance. For example, Top80 features in dataset M1105 consisted of 54 GO terms. There are only six cellular compartment GO terms, i.e., mitochondrial inner membrane GO:0005743, mitochondrial matrix GO:0005759, mitochondrial outer membrane GO:0005741, GO:0031307, GO:0045040, and GO:0005742. After removing the six GO terms from top80 features, we got an overall accuracy of 93.68%, which was still better than existing methods. Next, each individual feature type is removed from the integrated feature vector to test its prediction power. To facilitate the comparison, Top80 selected features from any two groups of features based on SVM-RFE are input to the classifier for evaluating the contribution of the missing feature type. We found that the prediction accuracy based on PSSM+GO features was 74.73% for M1105, which was slightly lower than that by PSSM+PROFEAT and PROFEAT+GO features (75.55% and 75.82%). We also test the performance of the prediction based only on one group of features (also top80 features). The respective accuracies based on PSSM, PROFEAT and GO features were 72.99%, 75.09% and 76.37%, which are also significantly lower than the overall accuracy by integration of all three types of features. For a human mitochondria protein dataset T86 with a small size, our method still achieved an overall accuracy of 100% (**Table 4**). The accuracies of the three subsets were 4.17%~13.33% improvements over the method constructed by Shi et al [27]. It is important to note that when M1105 dataset was used to calibrate the parameters, the accuracy at top80 was the highest for M1105. While that was top40 and top20 for the two smaller datasets M317 and T86. It could explain why the prediction accuracies for the two small test datasets reached 100% based on top80 features.

To further demonstrate the prediction power of our method, receiver operating characteristic (ROC) curves on three datasets were implemented here. However, protein submitochondria location prediction was a multi-class prediction problem. To address this problem, we first transformed protein submitochondria location prediction to multiple binary classifiers using *one-against-rest* strategy, and then averaged all the binary ROC curves as the final output of a method. **Figs. 4-6** showed the averaged ROC curves for three datasets by our method and the other three approaches. The area under curves (AUCs) of this method was 1 for all three datasets, which was significantly higher than those by PSSM, PROFEAT and GO features individually (e.g. AUCs were 0.8307, 0.8527 and 0.8547 for M1105, respectively).

3. Case study

To further illustrate our methods, we predicted the submitochondria locations of 11 proteins, most of which were related to colorectal cancer. As shown in **Table 5**, 10 of 11 proteins were correctly predicted to the right submitochondria locations by our predictor based on three datasets. For example, P00395 is a mitochondrion inner membrane protein, which is involved in colorectal cancer, a complex disease characterized by malignant lesions arising from the inner wall of the colon and the rectum [54]. In this study, it was consistently predicted as a mitochondrion inner membrane protein by our predictor on three datasets. Another example was Q9BRQ8, a mitochondrion outer membrane protein, which played an important role in EB1 colon cancer cells. Our predictor trained by all three datasets also correctly predicted it as a mitochondrion outer membrane protein. These results imply that our method is suitable for protein submitochondria location prediction.

Conclusions

In this work, an SVM-based model was constructed for the prediction of protein submitochondria localizations by selecting the optimal features from three kinds of important features, *i.e.*, protein GO function annotation, amino acid physical-chemical properties and PSI-BLAST profile. The prediction performance of our method for three low similarity datasets was very promising (99.37% for M317 and 100% for M1105 and T86). It supported the assumption that an optimal combination of multi-features could improve the prediction accuracies for protein submitochondria location prediction. Moreover, the recrusive feature extraction strategy adopted here was highly powerful in getting the optimal features, thus it accelerated the computing procedure as well as improved the final prediction results. The good performances of our predictor for evaluating different datasets suggest that our method is adaptable to diverse datasets and can be applied as a useful tool in such predicting tasks.

Admittedly, there are still some challenges need to be addressed in submitochondria localization prediction. Although our method suffered from a little high computational complexity for feature ranking and the inconsistent features chosen by different datasets, it could effectively catch the core features and improve the prediction of protein submitochondria location. In addition, we mainly focused on the proteins with single location sites. Since proteins with multiple location sites might play a significant role in cellular metalism, we will develop our method by addressing this problem.

Now that serviceable web-servers show the future direction for developing more useful methods, models and predictors [55, 56], in our future work, we will attempt to provide a web-server for this method.

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Competing interests

The authors declare that they have no competing interests.

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Table 1 The detailed information of three datasets in our predictor.

Submitochondria location	Number of proteins		
	M317	M1105	T86
Inner membrane	131	589	23
Outer membrane	41	236	15
Matrix	145	280	48
Total	317	1105	86

Table 2 Prediction performance comparisons by jackknife test for dataset M317.

Submitochondria locations	SUBMITO [23]		GP-LOC [24]		Predict_subMITO [8]		MitoLoc-LRSVM4 [25]		Method constructed by Fan and Li [26]		The proposed method	
	Accuracy (%)	MCC	Accuracy (%)	MCC	Accuracy (%)	MCC	Accuracy (%)	MCC	Accuracy (%)	MCC	Accuracy (%)	MCC
Inner membrane	85.50	0.79	83.21	0.80	91.8	0.79	89.31	0.84	94.70	0.91	100	0.99
Outer membrane	51.20	0.64	78.05	0.77	66.1	0.63	78.05	0.74	99.30	0.96	100	1.00
Matrix	94.50	0.78	97.24	0.85	96.4	0.79	93.79	0.87	80.50	0.84	98.61	0.99
Total accuracy	85.20	-	89.00	-	89.7	-	89.90	-	94.95	-	99.37	-

Table 3 Prediction performance comparisons by jackknife test for dataset M1105.

Submitochondria locations	Method constructed by Fan and Li [26]		The proposed method	
	Accuracy (%)	MCC	Accuracy (%)	MCC
Inner membrane	96.1	0.891	100.00	1.0000
Outer membrane	86.9	0.890	100.00	1.0000
Matrix	93.9	0.901	100.00	1.0000
Total accuracy	93.57	-	100.00	-

Table 4 Prediction performance comparisons by jackknife test for dataset T86.

Submitochondria locations	Method constructed by Shi and Qiu [27]		The proposed method	
	Accuracy (%)	MCC	Accuracy (%)	MCC
Inner membrane	86.96	0.7954	100.00	1.0000
Outer membrane	86.67	0.7427	100.00	1.0000
Matrix	95.83	0.8357	100.00	1.0000
Total accuracy	91.86	-	100.00	-

Table 5 Examples to show the predicted results by our predictor based on three datasets.

Accession number	Entry name	submitochondria location	The proposed method		
			Trained by M1105	Trained by M317	Trained by T86
P00395	COX1_HUMAN	Inner membrane	Inner membrane	Inner membrane	Inner membrane
Q9BRQ8	AIFM2_HUMAN	Outer membrane	Outer membrane	Outer membrane	Outer membrane
O14521	DHSD_HUMAN	Inner membrane	Inner membrane	Inner membrane	Inner membrane
P08074	CBR2_MOUSE	Matrix	Matrix	Matrix	Matrix
Q8IWA4	MFN1_HUMAN	Outer membrane	Outer membrane	Outer membrane	Outer membrane
O15239	NDUA1_HUMAN	Inner membrane	Inner membrane	Inner membrane	Inner membrane
P00156	CYB_HUMAN	Inner membrane	Inner membrane	Inner membrane	Inner membrane
P20000	ALDH2_BOVIN	Matrix	Matrix	Matrix	Matrix
Q96E52	OMA1_HUMAN	Inner membrane	Inner membrane	Inner membrane	Inner membrane
P22695	QCR2_HUMAN	Inner membrane	Outer membrane	Outer membrane	Matrix
P00403	COX2_HUMAN	Inner membrane	Inner membrane	Inner membrane	Inner membrane
Q969M1	TM40L_HUMAN	Outer membrane	Outer membrane	Outer membrane	Outer membrane

Figure legends

Figure 1. Top80 features in the three datasets.

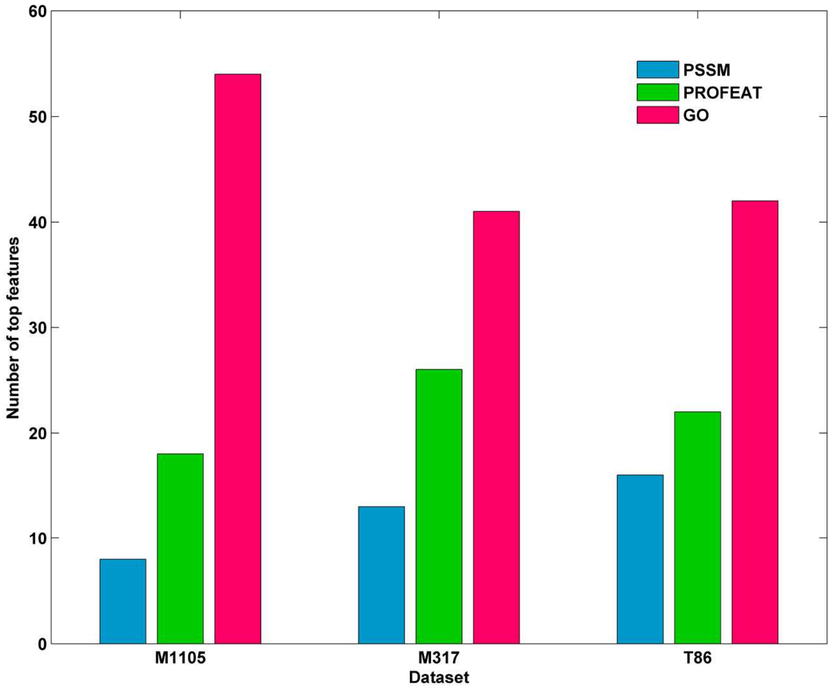
Figure 2. The pipeline that goes from the query sequence to the final output and all intermediate steps.

Figure 3. Comparison of prediction accuracies of different top features.

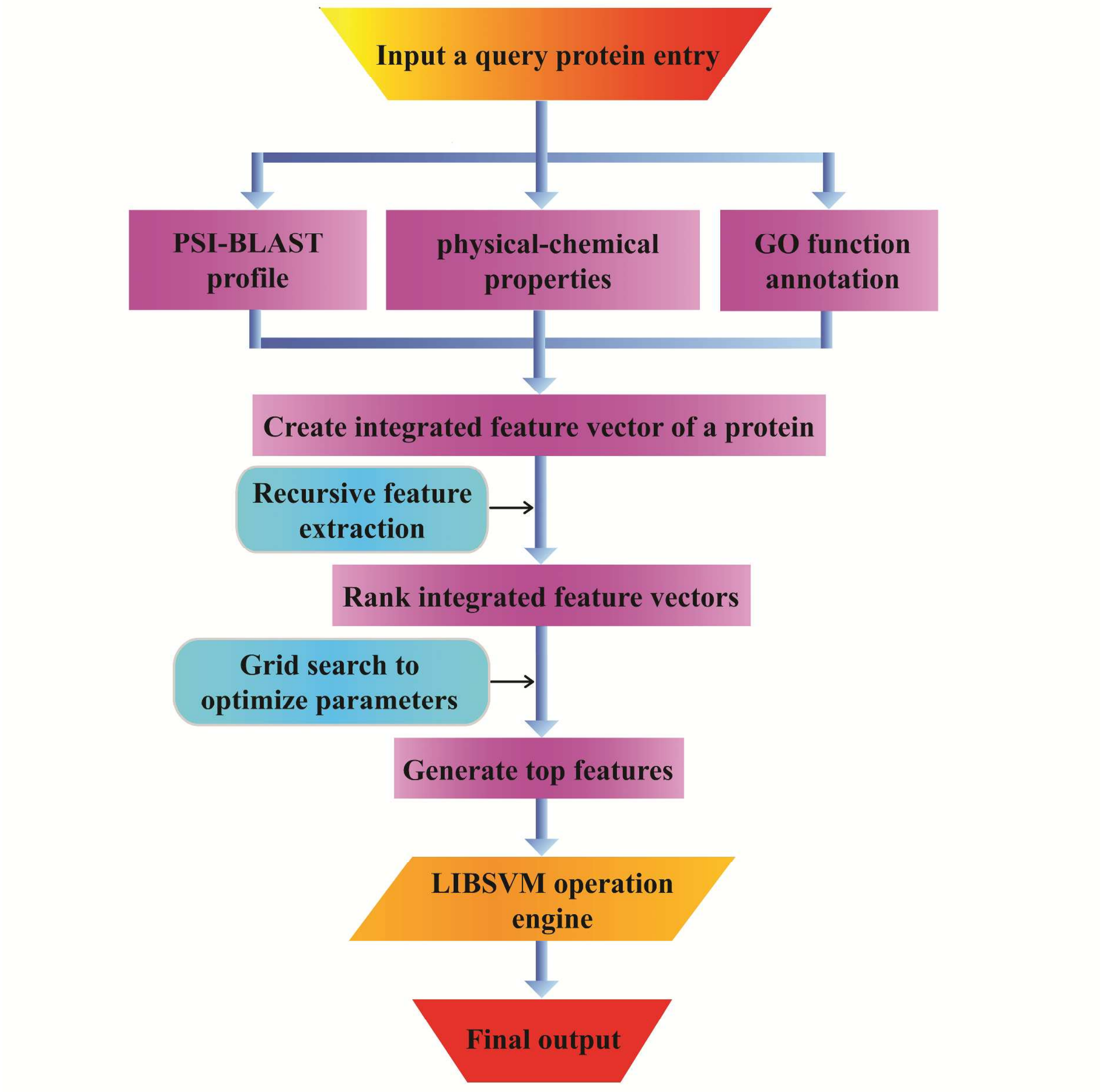
Figure 4. The ROC curves of M1105 dataset.

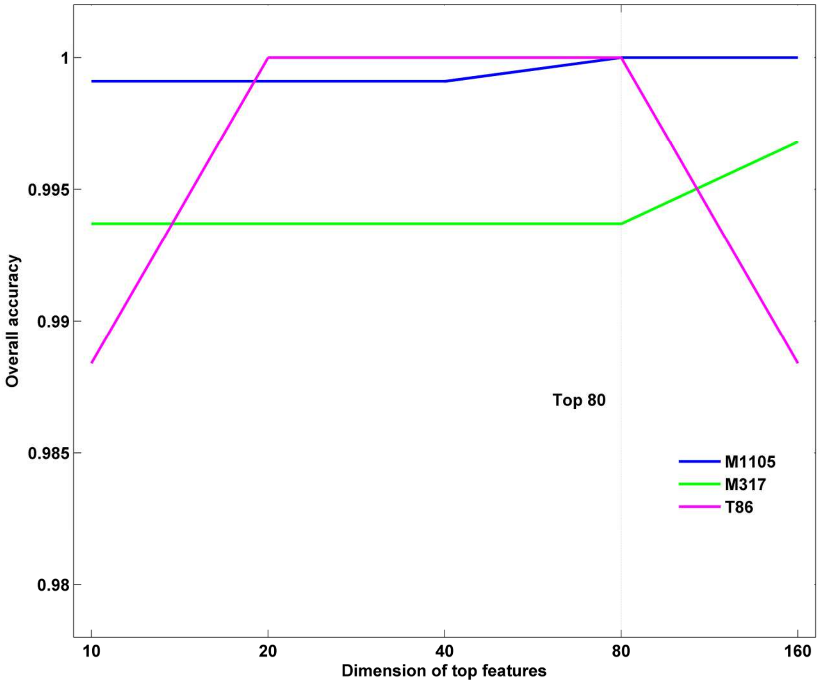
Figure 5. The ROC curves for M317 dataset.

Figure 6. The ROC curves for T86 dataset.

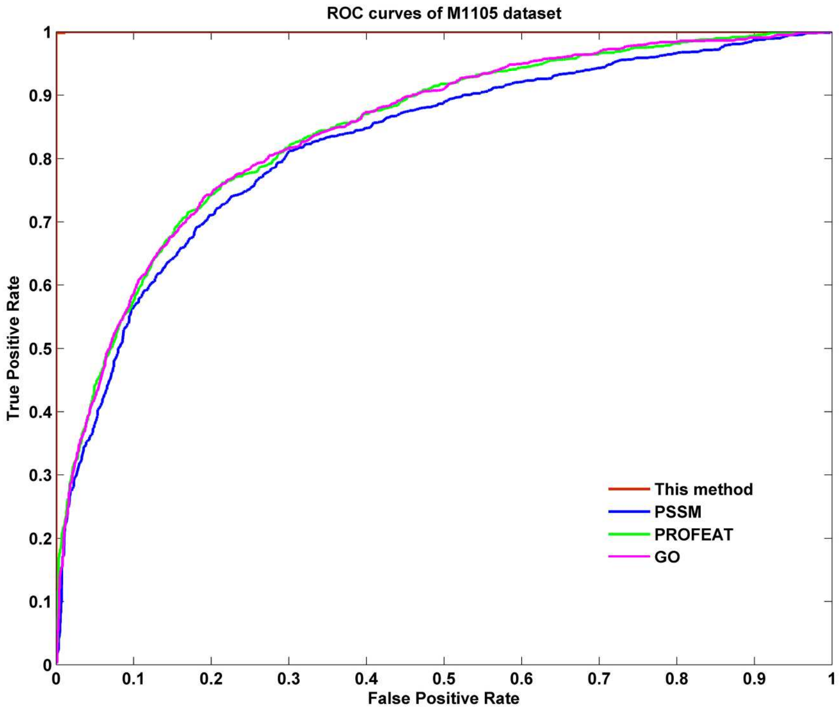


197x153mm (300 x 300 DPI)


Input a query protein entry
PSI-BLAST profile
physical-chemical properties
GO function annotation
Create integrated feature vector of a protein
Recursive feature extraction
Rank integrated feature vectors
Grid search to optimize parameters
Generate top features
LIBSVM operation engine
Final output



197x153mm (300 x 300 DPI)



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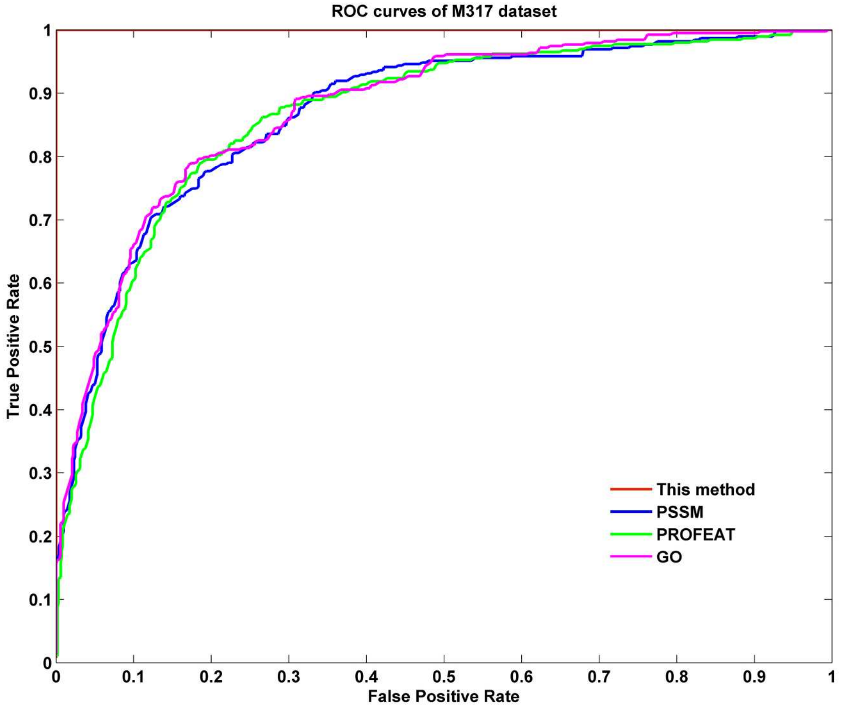
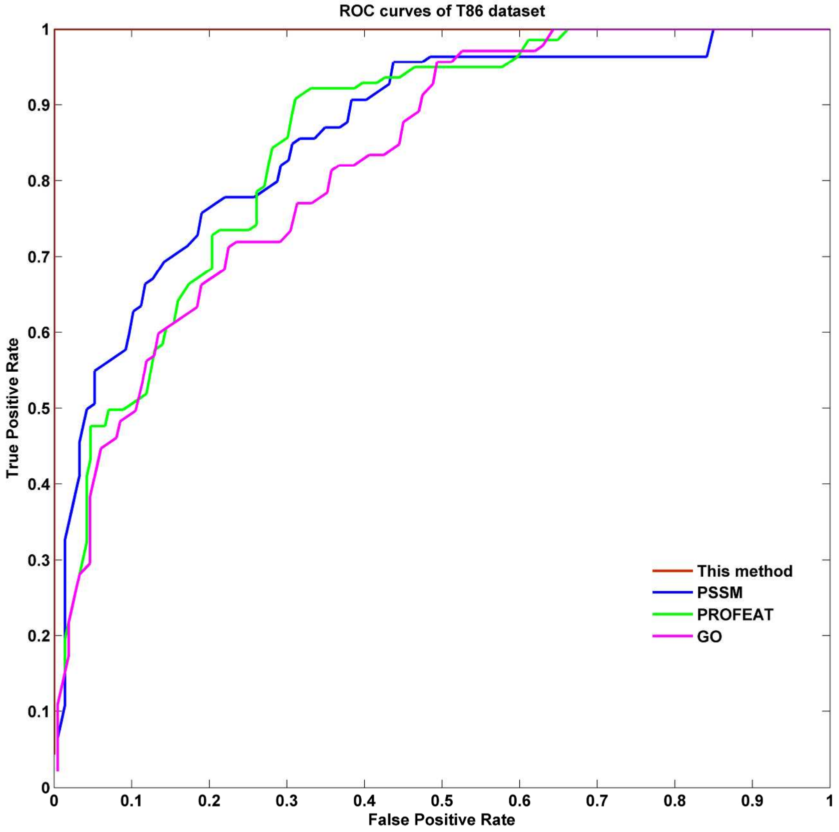


197x153mm (300 x 300 DPI)



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