Sequence analysis

Predicting the multi-label protein subcellular localization through multi-information fusion and MLSI dimensionality reduction based on MLFE classifier

Yushuang Liu$^{1,2}$, Shuping Jin$^{1,2}$, Hongli Gao$^{1,2}$, Xue Wang$^{1,2}$, Congjing Wang$^{1,2}$, Weifeng Zhou$^{1,2}$ and Bin Yu $^{3,4,*}$

$^1$College of Mathematics and Physics, Qingdao University of Science and Technology, Qingdao 266061, China, $^2$Artificial Intelligence and Biomedical Big Data Research Center, Qingdao University of Science and Technology, Qingdao 266061, China, $^3$School of Data Science, Qingdao University of Science and Technology, Qingdao 266061, China and $^4$College of Information Science and Technology, Qingdao University of Science and Technology, Qingdao 266061, China

*To whom correspondence should be addressed.

Abstract

Motivation: Multi-label (ML) protein subcellular localization (SCL) is an indispensable way to study protein function. It can locate a certain protein (such as the human transmembrane protein that promotes the invasion of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) or expression product at a specific location in a cell, which can provide a reference for clinical treatment of diseases such as coronavirus disease 2019 (COVID-19).

Results: The article proposes a novel method named ML-locMLFE. First of all, six feature extraction methods are adopted to obtain protein effective information. These methods include pseudo amino acid composition, encoding based on grouped weight, gene ontology, multi-scale continuous and discontinuous, residue probing transformation and evolutionary distance transformation. In the next part, we utilize the ML information latent semantic index method to avoid the interference of redundant information. In the end, ML learning with feature-induced labeling information enrichment is adopted to predict the ML protein SCL. The Gram-positive bacteria dataset is chosen as a training set, while the Gram-negative bacteria dataset, virus dataset, newPlant dataset and SARS-CoV-2 dataset as the test sets. The overall actual accuracy of the first four datasets are 99.23%, 93.82%, 93.24% and 96.72% by the leave-one-out cross validation. It is worth mentioning that the overall actual accuracy prediction result of our predictor on the SARS-CoV-2 dataset is 72.73%. The results indicate that the ML-locMLFE method has obvious advantages in predicting the SCL of ML protein, which provides new ideas for further research on the SCL of ML protein.

Availability and implementation: The source codes and datasets are publicly available at https://github.com/QUST-AIBBDRC/ML-locMLFE/.

Contact: yubin@qust.edu.cn.

Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

The structure and function of protein are various, but they can only play a role in the right subcellular localization (SCL) (Chu et al., 2020; Costa et al., 2018). When protein structure changes, it will cause diseases, such as kidney disease (Ivanova et al., 2008), myocarditis (Jang et al., 1988), diabetes (Brownlee, 1995), dermatomyositis (Brownlee, 1995) and muscle atrophy (Sneddon et al., 2000). With the continuous increase of data and the expansion of research directions (Wan et al., 2015, 2017), the traditional machine learning methods cannot achieve good prediction results (Marilyn et al., 2020; Zhang et al., 2020). First, the traditional machine learning methods are time-consuming and labor-intensive. Second, the protein not only exists in one SCL, but also may exist in two or multiple SCL. The prediction method for a single protein site ignores the situation of two or more subcellular locations (Du et al., 2020). Finally, the high-dimensional space formed by multi-information fusion increases the interference of redundant information on the prediction results (Yu et al., 2018). Therefore, this article mainly optimizes
the feature extraction, feature selection and classifier to improve the prediction accuracy.

Since protein sequences cannot be directly used for calculation, it must be transformed into digital information for further study (Yu et al., 2020a). Zhang et al. (2020b) utilized dipeptide composition (DC), pseudo position-specific scoring matrix (PsePSSM), pseudo amino acid composition (PseAAC), gene ontology (GO) and encoding based on grouped weight (EBGW) to extract protein information from relevant datasets. Wu et al. (2012) adopted GO and evolutionary information to develop a new predictor iLoc-Gps in Gram-negative bacteria dataset. Wan et al. (2014) used the relationship between GO terms to predict the SCL of plant dataset. Zhang et al. (2021b) used position-specific scoring matrix-transition probability composition (PSSM-TPC), DC, GO, PseAAC, PsePSSM and differential evolution algorithm to assign five single feature weight vector.

The feature fusion method can combine multiple information of protein sequences (Yu et al., 2021). But the interference of redundant information on the prediction results will gradually increase (Fan et al., 2021; Shu et al., 2019) with the increase of dimension. To eliminate the useless features in the original space, researchers have proposed a variety of dimensionality reduction methods. Zhang et al. (2019) put forward a manifold regularized discriminant feature selection (MDFS) algorithm to improve performance by optimizing feature selection framework and considering label correlation. Zhang and Zhou (2010) suggested a multi-label (ML) dimension reduction method based on dependency maximization (MDDM) to maximize the dependency of original features and related category labels to make the dimension reduction process more efficient. Xu et al. (2016) came up with the ML feature extraction algorithm via feature variance and feature-label dependence (MVMD) method, which integrated two least squares formulas and used the maximum feature variance and the correlation of feature label to select the best feature vector. Zhang et al. (2020a) presented global relevance and redundancy optimization (GRRO) method composed of feature relevance, label relevance and feature redundancy, which greatly improved computing efficiency.

Choosing a suitable classifier is crucial for predicting the SCL of proteins. Wan et al. (Wan and Mak, 2018; Wang et al., 2021) proposed an adaptive decision-making scheme for support vector machines (AD-SVM) to obtain the overall actual accuracy (OAA) on virus dataset was 93.24%, and the overall location accuracy (OLA) was 96.03%. Wang et al. (2015) used the ensemble multiple classifier chain (ECC) to predict the protein SCL of Gram-negative bacteria dataset, and the OAA was 94.03%, the OLA was 94.46%. Shen et al. (2019) used the multi-kernel SVM classifier to predict the two human datasets ML protein SCL, and the average precision reached 70.65% and 68.89%, respectively, compared with the results of other methods, the result was the best.

To improve the accuracy of prediction, we propose a new model called ML-locMLFE to predict the SCL of ML protein. Six feature extraction methods are used to transform protein sequences into digital information. Therefore, this article needs to fuse six types of feature information. Then we use the ML information latent semantic index (MLSI) to classify and recognize the most effective information from many features. Finally, the ML learning with feature induced labeling information enrichment (MLFE) is utilized to predict the SCL of ML protein. Compared with other methods, ML-locMLFE is more superior in predicting the SCL of ML protein.

2 Materials and methods

2.1 Datasets

Five datasets are used to verify the effectiveness of the model. The Gram-positive bacteria dataset (Dehzangi et al., 2015) is the training set, while the Gram-negative bacteria dataset (Dehzangi et al., 2015), the virus dataset (Shen and Chou, 2010), the SARS-CoV-2 dataset (Zhang et al., 2020b) and the newPlant dataset (Wan et al., 2012) are the test sets together. The Gram-positive bacteria dataset, Gram-negative bacteria dataset, virus dataset come from the Swiss-Prot database and the breakdown of each dataset is shown in Supplementary Tables S1–S3. We have obtained data from the UniProt database of the past 3 years to construct a new plant dataset (named as the newPlant dataset). The detailed breakdown is given in Supplementary Table S4. As a newly mutated coronavirus, the SARS-CoV-2 can cause great harm to human health. Therefore, the accurate identification of the SCL of the SARS-CoV-2 protein is helpful to analyze the pathogenic mechanism of the virus. The SARS-CoV-2 dataset is constructed from the UniProt database, and the detailed breakdown is shown in Supplementary Table S3. The homology of the five datasets is <25%.

2.2 Feature encoding

The quality of features has a crucial impact on the predictive ability of the model. Therefore, a suitable feature extraction method is an extremely critical step in predicting the SCL of ML protein. Six methods, namely PseAAC, EBGW, GO, residue probing transformation (RPT), evolutionary characteristic transformation (EDT) and multi-scale continuous and discontinuous (MCD), are adopted here.

PseAAC: PseAAC is a commonly used feature extraction method to predict SCL. According to Chou (2001), PseAAC mainly reflects the protein sequence information (Bahar et al., 1997; Sahu et al., 2020; Zhang et al., 2021a). The algorithm can be expressed by

\[
L = [l_1, l_2, \ldots, l_{20}, l_{20+1}, \ldots, l_{20+\beta}]^T
\]

where \(l_i\) represents the level sequence correlation factor, \(f_v\) represents the frequency of the \(v\)th amino acid in the protein, \(\omega_i\) is the weighting factor and the value selected in this article is 0.05 (Chou, 2001). Because \(\beta\) is the characteristic parameter, a \(20 + \beta\)-dimensional feature vector will be formed finally.

EBGW: The physical and chemical properties is one of the important properties of protein. Zhang et al. (2006) proposed EBGW, which divided amino acids into four categories, as shown in Table 1.

| Group              | Amino acids |
|--------------------|-------------|
| Neutral and hydrophobic amino acids (K1) | A, F, G, I, L, M, P, V, W |
| Neutral and polarity amino acids (K2) | C, N, Q, S, T, Y |
| Acidic amino acids (K3) | K, H, R |
| Alkaline amino acids (K4) | D, E |

Three disjoint combinations can be obtained from Table 1. According to formulas (3), (4) and (5), the protein sequences are converted into three binary sequences.

\[
f_1(p_i) = \begin{cases} 1 & p_i \in \{K1, K2\} \\ 0 & p_i \in \{K3, K4\} \end{cases}
\]

\[
f_2(p_i) = \begin{cases} 1 & p_i \in \{K1, K3\} \\ 0 & p_i \in \{K2, K4\} \end{cases}
\]

\[
f_3(p_i) = \begin{cases} 1 & p_i \in \{K1, K4\} \\ 0 & p_i \in \{K2, K3\} \end{cases}
\]

The length of three binary sequences is \(L\). These sequences are divided into multiple subsequences and the subsequence length is progressive increase. Each will form \(L\)-dimensional feature vector, so three binary sequences form \(3 \times 1\)-dimensional vector.

GO: When using GO model to extract GO information of each protein sequence, it is usually divided into two steps (Huang et al., 2008; Shen et al., 2020). One is GO terms, and another is GO
vector. The BLASTP is used to search from the Swiss-Prot database and retain homologous proteins (denoted as \( Y_i \)) with a similarity \( \geq 60\% \) with protein \( P_r \). Parameter \( E \) is set to 0.001 (Zhang et al., 2018a) in the above steps. In the GOA database, we searched for accession number (ACs) of each protein in \( Y_i \), which are obtained from the Swiss-Prot database. Then, the corresponding GO terms were obtained (Xiao et al., 2011). Then, GO feature vector is constructed as:

\[
p_i = [G_1, G_2, \ldots, G_{|Y_i|}]^T \quad i = 1, 2, \ldots, |Y_i| \tag{6}
\]

Here, \( |Y_i| \) is the size of \( Y_i \) dataset, \( G_{|Y_i|} = \begin{cases} 1, & p_i \in G_{|Y_i|} \\ 0, & \text{otherwise} \end{cases} \)

RPT: RPT is a feature extraction method that reflects the evolutionary information of protein sequences (Jeong et al., 2010). In the PSSM, domains with similar conservations are grouped according to conservation scores (Wang et al., 2019; Zhang et al., 2021c). Here, each particular columns corresponding are standard amino acids in the PSSM. The 20 amino acids are separated 20 groups as rows in each particular columns corresponding are standard amino acids in the PSSM. The 20 amino acids are separated 20 groups as rows in each particular columns corresponding are standard amino acids in the PSSM. The 20 amino acids are separated 20 groups as rows in each particular columns corresponding are standard amino acids in the PSSM.

\[
RPT = \begin{pmatrix} H_{1,1} & H_{1,2} & \cdots & H_{1,20} \\
H_{2,1} & H_{2,2} & \cdots & H_{2,20} \\
\vdots & \vdots & \ddots & \vdots \\
H_{20,1} & H_{20,2} & \cdots & H_{20,20} 
\end{pmatrix}
\tag{7}
\]

Therefore, the matrix can be transformed into a 400-dimensional feature vector \( V = [b_{i_1}, b_{i_2}, \ldots, b_{i_m}, \ldots, b_{i_{20}}] \), where \( b_{i_m} \) is obtained by equation (8).

\[
b_{i_m} = \frac{H_{im}}{L} \quad (n, m = 1, 2, \ldots, 20) \tag{8}
\]

EDT: EDT is an effective method to calculate the non-occurrence information possibility of two amino acids (Jeong et al., 2010). The two amino acids with interval of \( d(1, 2, \ldots, L_{\text{min}} - 1) \), where \( L_{\text{min}} \) is the shortest sequence length in the dataset. The feature vector of EDT is denoted as:

\[
P = (f(A_1, A_1), f(A_1, A_2), \ldots, f(A_1, A_{20}), \ldots, f(A_n, A_1), f(A_n, A_2), \ldots, f(A_n, A_{20}))
\tag{9}
\]

The \( f(A_x, A_y) \) is non-occurrence possibility of two amino acids with interval \( d \). It is calculated by formula (10):

\[
f(A_x, A_y) = \frac{1}{d-1} \sum_{i=1}^{d-1} (A_{x_i} - A_{y_{i+d}})^2 \tag{10}
\]

where \( A_{x_i}, A_{y_{i+d}} \) are the elements in the PSSM, \( A_x, A_y \) are any 2 of the 20 amino acids, \( D \) is the maximum value in \( d \).

MCD: Due to the influence factors of continuous and discontinuous fragments in protein sequences, You et al. (2014) proposed the MCD feature extraction method. The method converts protein sequence into digital information by binary method. For example, a protein sequence ‘AVDCALSK’ is randomly selected and transformed into a digital model ‘11321476’ via MCD calculation. Then, the sequence is divided into 10 regions, thus composition (C), transition (T) and distribution (D) are used to represent protein characteristics and each descriptor can be calculated. Finally, a 630-dimensional feature vector is formed by all descriptors from 10 regions.

2.3 ML informed latent semantic indexing

Assuming the feature space contains \( N \) samples, and each sample size is \( M \)-dimensional feature vector, but we will reduce to \( L \) dimension. MLSI (Yu et al., 2005) defines the input matrix \( X = [x_1, x_2, \ldots, x_N] \in \mathbb{R}^{N \times M} \), where \( x_i \) is the \( M \)-dimensional feature vector. The output matrix \( Y = [y_1, y_2, \ldots, y_N] \in \mathbb{R}^{N \times L} \) and \( y_i \) is the \( L \)-dimensional feature vector. Kernel function \( k_x(\cdot, \cdot) \) represents inner product as:

\[
k_x(x_i, x_j) = \langle x_i, x_j \rangle \tag{11}
\]

Similar kernel function \( k_y(\cdot, \cdot) \) is expressed as equation (12), and the kernel matrix \( K_y = YY^T \) is obtained.

\[
k_y(y_i, y_j) = \langle y_i, y_j \rangle \tag{12}
\]

The kernel calculation matrix \( C \) is as (13):

\[
C = (1 - \beta)K_x + \beta K_y \tag{13}
\]

Then, for generalized eigenvalue problems,

\[
[K_x C^{-1} + \gamma K_y]x = \lambda K_y x \tag{14}
\]

where coefficient \( \lambda \) requires \( \lambda^2 K_y x = 1, \lambda_1 \leq \cdots \leq \lambda_N \). By formula (14), the generalized eigenvalues \( \lambda_1, \cdots, \lambda_N \) are calculated and the first \( K \) eigenvalues are used as mappings. The \( \eta \)-mapping function can be obtained by scaling the eigenvalues:

\[
\varphi_x(x) = \frac{1}{\sqrt{\lambda_i}} w_i^T x = \frac{1}{\sqrt{\lambda_i}} \sum_{j=1}^{N} (x_j) k_y(x_i, x) \tag{15}
\]

Then, \( \lambda = 1/\hat{\lambda} \) and formula (15) is rewritten as:

\[
K_y^2 x = \hat{\lambda} K_x C^{-1} K_y + \gamma K_y x \tag{16}
\]

Finally, \( k \)-dimensional vector with the largest eigenvalue is selected.

2.4 ML learning with feature induced labeling information enrichment

If training sample is denoted as \( \langle x_i, y_i \rangle \), \( p \) is the number of training sample, and given the enriched labeling information \( U \), the original training sample can be transformed into \( D = \{ (x_i, u_i) \mid 1 \leq i \leq p \} \). The response variables \( u_i \) can measure the model through the multi-output regression technology. MLFE algorithm (Zhang et al., 2018b) uses minimization to obtain the objective function of the regression model:

\[
\Omega(\Theta, \beta) = \sum_{i=1}^{p} \Omega_i(u_i) + \beta \sum_{i=1}^{p} \sum_{j=1}^{q} \Omega_2(o_{ij}) + \beta \sum_{i=1}^{p} \sum_{j=1}^{q} \Omega_3(o_{ij}) + \frac{1}{2} \sum_{l=1}^{m} \| \theta_l \|_2^2 \tag{17}
\]

where \( \Theta = [\theta_1, \theta_2, \ldots, \theta_q] \) and \( b = [b_1, b_2, \ldots, b_q]^T \) represent weight matrix and deviation vector of regression model, respectively, and \( q \) is the number of class label.

To obtain the optimal objective function, Newton-weighted least squares iterative method (IRWLS) (Sanchez-Fernández et al., 2004; Tsoumakas et al., 2011) is used. In the iterative process, the descent direction of model optimization is determined by solving the linear solution of the equation. Let \( \{ \Theta^{(k)}, b^{(k)} \} \) represents the current model after the \( k \)-th iteration, and equation (18) is obtained based on the first-order Taylor expansion.

\[
\Omega'(\Theta, \beta) = \beta_1 \sum_{i=1}^{p} \Omega_1(u_i) + \beta_1 \sum_{i=1}^{p} \frac{\partial \Omega_1(u_i)}{\partial u_{ik}} (e_i^{(k)})^T (e_i - e_i^{(k)}) + \beta_1 \sum_{i=1}^{p} \sum_{j=1}^{q} \Omega_2(o_{ij}) + \beta_1 \sum_{i=1}^{p} \sum_{j=1}^{q} \Omega_3(o_{ij}) + \frac{1}{2} \sum_{l=1}^{m} \| \theta_l \|_2^2 \tag{18}
\]
direction, it is necessary to construct the quadratic approximation value of $d\Omega(t)/u$:

$$
\Omega'(\Theta, b) = \beta_1 \sum_{i=1}^{p} \Omega_1(u_i^{(k)}) + \frac{d\Omega_1(u_i^{(k)})}{du_i^{(k)}} u_i^{(k)} - \frac{1}{2} (\beta_1)^2 
+ \beta_2 \sum_{i=1}^{p} \sum_{j=1}^{q} \Omega_2(x_{ij}) + \beta_3 \sum_{i=1}^{p} \sum_{j=1}^{q} \Omega_3(x_{ij}) + \frac{1}{2} \sum_{i=1}^{p} \|\theta_i\|^2_2 + Y
$$

where $Y$ is a constant term.

2.5 Performance evaluation

The cross-validation method can avoid over-fitting to some extent. The commonly used methods include $K$-fold cross-validation (Jia et al., 2018), leave-one-out cross validation (LOOCV) (Yu et al., 2020b), self-compatibility method (Bringi et al., 2001) and independent sample test (Heeren and D’Agostino, 1987). Compared with other cross-validation methods, LOOCV is deterministic and has high sample utilization (Cheng et al., 2017). Therefore, the LOOCV test is introduced in this article to evaluate the effectiveness of the model with the OAA, OLA, hamming loss (HL), coverage (CV), ranking loss (RL), and average precision (AP) as indicators. Six evaluation indicators are defined as the following.

$$
OAA = \frac{1}{W} \sum_{i=1}^{W} |Y_i(U_i) \cap Y_i'(U_i)|
$$

$$
OLA = \frac{1}{W} \sum_{i=1}^{W} |Y_i(U_i) \cap Y_i'(U_i)|
$$

where $W$ is the number of training sample. $Y_i(U_i)$ and $Y_i'(U_i)$ represent prediction label and real label, $\Delta[Y_i(U_i), Y_i'(U_i)] = \{1$, $Y_i(U_i) = Y_i'(U_i)$; $0$, otherwise $\}$.

$$
HL = \frac{1}{W} \sum_{i=1}^{W} (Y_i(U_i) \cup Y_i'(U_i)) - (Y_i(U_i) \cap Y_i'(U_i))
$$

where $G$ is the number of labels.

$$
CV = \frac{1}{W} \sum_{i=1}^{W} \text{rank}(f(X_i, y)) - 1
$$

where $\text{rank}(f(X_i, y)) - 1$ makes all labels rank down and get the corresponding ranking.

$$
RL = \frac{1}{W} \sum_{i=1}^{W} \frac{1}{|Y_i|} |\text{RAL}(X_i)|
$$

where $\text{RAL}(X_i) = \{(y_j, y_k) \mid f(X_i, y_j) \leq f(X_i, y_k), (y_j, y_k) \in Y_i \times Y_i\}$ and $y_j \in Y$. $f(X_i, y)$ is part of the label of $X_i$, $\overline{Y}$ is a supplement of $Y_i$.

$$
AP = \frac{1}{W} \sum_{i=1}^{W} \frac{1}{|Y_i|} \sum_{y_j \in Y_i} \text{AVP}(T_{y_j})
$$

where $\text{AVP}(T_{y_j}) = \frac{|y_j \cap \text{rank}(T_{y_j})|}{\text{rank}(T_{y_j})}$.

This study proposes a new method ML-locMLFE for predicting the SCL of ML proteins and the detailed process is displayed in Figure 1.

![Flowchart of ML-locMLFE prediction method](image)

**Fig. 1.** Flowchart of ML-locMLFE prediction method. (i) Data preparation. Five datasets are obtained from Swiss-Prot and UniProtKB databases, then the corresponding protein sequences and real label are also achieved. (ii) Feature extraction. PseAAC, EBGW, MCD, RPT, EDT and GO are used to convert protein sequence information into digital information, then the six features vector are fused. (iii) Feature selection. The MLSI method identifies the most effective information to form the optimal feature subset. (iv) Model construction. Combining step (iii), the optimal feature subset is integrated into the classifier MLFE, and the ML-locMLFE model is constructed based on LOOCV. (v) Model evaluation. Gram-positive bacteria dataset is used to evaluate the effectiveness of ML-locMLFE, and Gram-negative bacteria dataset, virus dataset, SARS-CoV-2 dataset, newPlant dataset are used to verify the performance of ML-locMLFE. Both the training set and the test sets will choose OAA, OLA, HL, AP and CV as evaluation indicators.

![OAA obtained by Gram-positive bacteria dataset under different parameters.](image)

**Fig. 2.** The OAA obtained by Gram-positive bacteria dataset under different parameters. (A) The OAA reaches its maximum at $\beta = 25$, therefore, $\beta = 25$ is selected as the optimal parameter of PseAAC and forms a 20 + 120 = 140 dimensional vector. (B) The OAA reaches its maximum at $L = 40$, therefore, we select $L = 40$ as the best parameter of EBGW and form a $3 \times L = 120$ dimensional vector.

3 Results

3.1 The result analysis of feature encoding parameters $\beta$ and $L$

PseAAC and EBGW have different characteristic information by setting different parameters. Since the minimum length of all protein sequences of the Gram-positive bacteria dataset is 55, the parameter of PseAAC is set from 5 to 54 and the parameter of EBGW is set from 5 to 55. Through the LOOCV test, the characteristic information obtained from each parameter is put into the classifier MLFE, and the specific evaluation index values of the different parameter results are listed in the Supplementary Tables S6 and S7. The optimal OAA obtained from PseAAC and EBGW are 62.44% and
58.96%. The comparison results under different parameters are shown in Figure 2.

3.2 The influence of feature extraction methods on results
This article uses a total of six feature extraction methods. Among them, the PseAAC method not only considers the sequence information of the protein but also includes the position information of the amino acids in the sequence. The EBGW method is based on the physical and chemical properties of amino acids to effectively extract the physical and chemical information of proteins. The MCD method uses multiple regions as features to extract the physical and chemical information of protein sequences. The GO method extracts the annotation information of the protein, which can essentially analyze the properties of genes and gene products. Because the EDT method considers the evolutionary information of the protein, it can reflect the probability of two different amino acids. The RPT method obtains the evolutionary information of the protein by grouping the evolution scores in FSSM. Therefore, the six feature extraction methods obtain effective information from the different characteristics of the protein, which greatly improves the prediction performance of the model. Through the LOOCV test, six single feature vectors are put into MLFE, and GO has the largest contribution rate among all single features, and its OAA and OLA reach 91.91% and 93.31%, respectively. However, single feature information cannot represent all important information. Therefore, the six feature extraction results need to be fused. We extract 912-dimensional feature vectors from GO, 45-dimensional feature vectors from PseAAC, 120-dimensional feature vectors from EBGW, 400-dimensional feature vectors from RPT and EDT, respectively, and 630-dimensional feature vectors from MCD. After the final fusion, 2507-dimensional feature vectors are obtained. Through the LOOCV test, the comparison results of single and fusion features are given in Figure 3.

3.3 Analysis of feature selection results
Feature selection method can reduce spatial dimensions and decrease model training time. Therefore, this article uses principal component analysis (PCA) (Abdi and Williams, 2010), GRRO (Zhang et al., 2020a,b,c), MDFS (Zhang et al., 2019), MDDM (Zhang and Zhou, 2010), MVMD (Xu et al., 2016), MLSE (Yu et al., 2005) to eliminate irrelevant features. Through LOOCV test, the feature subset obtained by each method is put into MLFE. Then, the OAA of MLSE reaches 99.23%, and the OLA reaches 99.81%, which are both optimal. The algorithm not only retains the original input features, but also captures the correlation of output dimensions, which greatly improves the performance of model prediction. On the Gram-positive bacteria dataset, the MLSE method selects different dimensions to obtain the prediction results which are shown in Supplementary Table S8, and the comparison results of different methods can be found in Figure 4.

3.4 Comparative analysis of classification algorithms
To verify the effectiveness of MLFE, we take five classifiers as comparison. That are ML k-nearest neighbor (ML-KNN) (Gonzalez-Lopez et al., 2018), ML radial basis function (ML-RBF) (Zhang, 2009), ML learning with label-specific features (LIFT) (Zhang and Wu, 2015), ranking SVM (Rank-SVM) (Tayal et al., 2018), ML learning by instance differentiation (INSDIF) (Zhang et al., 2007). The optimal feature subset obtained by the MLSE is put into six classifiers. Through the LOOCV test, the results of OAA and OLA obtained from the MLFE classifier are 99.23% and 99.81%, respectively. The algorithm uses the sparse reconstruction information between the training samples as features, and the reconstruction information is passed into the label space to enrich the original labels as numerical labels, thereby enhancing the effectiveness of the label information. The comparison results of different methods are shown in Figure 5, and the corresponding receiver operating characteristic (ROC) and precision-recall (PR) curves are shown in Figure 6. The specific parameter values obtained through different algorithms are shown in Supplementary Table S9.
3.5 Comparison with other methods

With the continuous development of ML protein SCL research, many researchers use machine learning methods to predict. To prove the superiority of the ML-locMLFE, we compare the results of four datasets with other methods. On the Gram-positive dataset, the results of this article are compared with the results of Loc-pos (Wu et al., 2012), Gpos-ECC-mPLoc (Wang et al., 2015) and Gram-locEN (Wan et al., 2017). The results of different methods are listed in Figure 7. On the Gram-negative dataset, the results of this article are compared with the results of iLoc-Gneg (Chou and Shen, 2006), Gneg-ECC-mPLoc (Wang et al., 2015) and Gram-locEN (Wan et al., 2017). On the virus dataset, the results of this article are compared with the results of mGOASVM (Wan et al., 2012), AD-SVM (Wan and Mak, 2018) and mPLR-Loc (Wan et al., 2015). On the newPlant dataset, the results of this article are compared with the results of Plant-mPLoc (Chou and Shen, 2010), mPLR-Loc (Wan et al., 2015) and HybridGO-Loc (Wan et al., 2014). The result of different comparison on the newPlant dataset is shown in Table 2. (Hoffmann et al., 2020) found that SARS-CoV-2 transmission depended on transmembrane protease serine 2 (TMPRSS2), the protease inhibitors of TMPRSS2 can block SARS-CoV-2 into cells. Xu et al. (2020) predicted that the TMPRSS2 can bind to some monomeric compounds independently by studying the protein properties and 3D structure of TMPRSS2. Therefore, it is shown that the TMPRSS2 is a serine protease anchored on the cell membrane at the amino-terminal transmembrane region, and its inhibitor can be used as the treatment of COVID-19. The SARS-CoV-2 protein information is obtained by PseAAC, EBGW, GO, RPT, EDT and MCD to form the original feature space. With the continuous increase of the dimensionality, the interference of redundant information on the result is gradually significant. Thus, we use MLSI to obtain the optimal feature subset. Using the MLFE algorithm, the OAA is 72.73% and the OLA is 69.23%. The specific result comparison is shown in Table 2. Table 2 shows that the SARS-CoV-2 protein mainly exists in Plasma membrane, nucleus, Golgi apparatus and other subcellular. We obtained 26 proteins from the UniProt database and found that the ninth protein is TMPRSS2, which is located on the plasma membrane and accurately predicted by ML-locMLFE. The SARS-CoV-2 dataset is too small to optimize the model, the stability of the model is relatively low. Therefore, the prediction results of Cytoskeleton, Endoplasmic reticulum, Endosome, Golgi apparatus and Lysosome are not ideal, but the OAA and OLA are 72.73% and 69.23% by the ML-locMLFE method. The model presented in this article can not only predict the SCL of important protein in the SARS-CoV-2 protein quickly and accurately, but also provide a theoretical basis for the treatment of SARS-CoV-2 pneumonia and drug research.

Table 2. Prediction of protein SCL using ML-locMLFE on SARS-CoV-2 dataset

| Locations           | ML-locMLFE |
|---------------------|------------|
| Plasma membrane     | 10/10 = 1.0000 |
| Cytoskeleton        | 0/1 = 0.0000 |
| Endoplasmic reticulum | 0/1 = 0.0000 |
| Endosome            | 0/2 = 0.0000 |
| Golgi apparatus     | 0/1 = 0.0000 |
| Lysosome            | 0/2 = 0.0000 |
| Mitochondron        | 0/2 = 0.0000 |
| Nucleus             | 7/7 = 1.0000 |

4 Conclusion

It is significant to understand the structure and function of protein by using machine learning methods to predict the SCL of ML protein. First, PseAAC, EBGW, GO, RPT, EDT, MCD are used to extract important information about various properties of proteins. Among them, the GO method has the highest prediction accuracy and the largest contribution rate compared with the other five methods. The annotation information of genes and gene products extracted using the GO method can provide important evidence for the study of protein functions. Second, it is the first time to use MLSI as feature selection in the prediction of ML protein SCL. This method can map input features to a new feature space, which not only ensures the existence of input information, but also captures the correlation between multiple output information, so that it can more effectively select the best feature subset. Finally, we integrate the optimal feature subset into MLFE. For the first time, MLFE algorithm is used to enrich the original labels of training samples into numerical labels to enhance the effectiveness of ML information, which further improves the performance of the model. Through the LOOCV test, the OAA of the Gram-positive bacteria dataset, the Gram-negative bacteria dataset, the virus dataset, the SARS-CoV-2 dataset and the newPlant dataset are 99.23%, 93.82%, 93.24%, 72.73%, 96.72%, and the OLA are 99.81%, 96.50%, 99.21%, 69.23%, 96.25%, respectively. Therefore, the ML-locMLFE proposed in this article can predict the ML protein SCL more accurately. In addition, the ML-locMLFE model can spread to other research...
fields such as ML protein post-translational modification, ML mRNA SCL and identification of drug-target interactions. More importantly, the model can accurately predict the SCL of the SARS-CoV-2 protein, and then clarify the pathogenic mechanism of the virus. We hope that our method can provide some insights and help in the clinical treatment of various diseases, including COVID-19. In the next step, we will construct larger scale and diverse datasets to study the SCL of ML protein.

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