Prediction of Multi-Type Membrane Proteins in Human by an Integrated Approach

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Abstract

Membrane proteins were found to be involved in various cellular processes performing various important functions, which are mainly associated to their types. However, it is very time-consuming and expensive for traditional biophysical methods to identify membrane protein types. Although some computational tools predicting membrane protein types have been developed, most of them can only recognize one kind of type. Therefore, they are not as effective as one membrane protein can have several types at the same time. To our knowledge, few methods handling multiple types of membrane proteins were reported. In this study, we proposed an integrated approach to predict multiple types of membrane proteins by employing sequence homology and protein-protein interaction network. As a result, the prediction accuracies reached 87.65%, 81.39% and 70.79%, respectively, by the leave-one-out test on three datasets. It outperformed the nearest neighbor algorithm adopting pseudo amino acid composition. The method is anticipated to be an alternative tool for identifying membrane protein types. New metrics for evaluating performances of methods dealing with multi-label problems were also presented. The program of the method is available upon request.

Introduction

Membrane proteins are one of the three main protein classes. It is approximately estimated that 20-30% of all genes in most genomes encode membrane proteins [1]. Gao et al. [2] estimated the number of membrane proteins was about 8000 in human. Membrane proteins also play wide varieties of important roles involved cellular processes [3], in the immune response, serve as enzymes, etc. In addition, membrane proteins constitute 60% of drug targets [4], which were crucial to new drug discovery as well as to understand the mechanism of the cellular activities [4,5,6]. It is reported that functions of a membrane protein are closely associated with its type [7]. However, it is time-consuming and costly to determine types of uncharacterized membrane proteins by using traditional biophysical methods [8]. Thus, there is a growing need for effective computational methods to predict the membrane protein types.

In the past decades, it was considered the importance of the predictions. Many tools were developed based on machine learning techniques, in which both features extracted from samples and the learning algorithms were equally affecting. Features used for the predictions were mainly including: amino acid composition (AAC), sequence homology information such as position-specific scoring matrices (PSSMs), pseudo amino acid composition (PseAAC), physicochemical properties of amino acids and functional domains. AAC was the simplest but often the most efficient to represent protein sequence. Cai et al. [9] used only AAC feature by using support vector machine (SVM) to predict membrane protein types. Reflecting the order of amino acid in the sequence and complementing AAC, PseAAC of protein sequence is considered to be the extension of it. Those features were widely applied to recognize types of uncharacterized membrane proteins [7,10,11,12,13]. Wang et al. [14], however, pointed out that the feature space of PseAAC was redundant, and they utilized a Supervised Locally Linear Embedding (SLLE) algorithm [15] for nonlinear dimensionality reduction. Pu et al. [16] proposed IAMPC, in which they extracted features from both PSSMs and protein primary sequences by using a so-called SVM fusion to identify membrane proteins types. Hayat et al. [17] used both the split amino acid composition (SAAC) and seven different physicochemical properties of proteins to represent protein sequences. They also employed SVM as the classifier. The method has been implemented in a web server called Mem-PHybrid (http://111.68.99.218/Mem-PHybrid). The hybrid fea-
tutes of PSSM and the SAAC of protein sequences were also used by Hayat et al. [18] to construct a web-based predictor. Ding et al. [19] used tripeptide compositions to recognize mycobacterial membrane protein types. Jia et al. [9] constructed a classifier based on hybrid feature space of domain profiles and physiochemical properties of membrane proteins. Hayat et al. [20] used features of split amino acid as well as the ensemble classifier. Chen et al. [21] suggested novel multiple-source features such as transmembrane segments, lipid-binding domains, signal peptides, signal anchors, GPI-anchoring signals, surface amino acids, cationic patches, and so on. On the other hand, some machine learning algorithms have been applied for the prediction of membrane protein types. For example, Fourier spectrum [22,23], wavelet analysis and cascaded neural network [24], wavelet transform (DWT) combined with SVM [25], neighborhood preserving embedding algorithm and K-nearest neighbor algorithm [26]. However, most of these methods did not consider the fact that one membrane protein possesses multiple types, which is common for membrane proteins.

Sequence homology reflects evolutionary relationships, which in turn helps infer structures and functions of proteins. Large bodies of protein-protein interactions available provide global perspective on relationship between different proteins at system-level. It is reported that proteins share identical properties and functions with interactive proteins more probably than with none-ones. Therefore, it is possible to identify membrane protein types by means of these. In this study, we proposed an integrated method by using both the homologies between protein sequences and the similar properties between interactive proteins to predict multi-types of membrane proteins in human. To widely examine the method, three datasets were constructed from UniProt database [27]. It was suggested from the performance of the method that it could be quite effective to identify membrane protein types.

Materials and Methods

Datasets

3,789 sequences of experimentally verified membrane proteins of human were downloaded from the UniProt database [27]. According to their intramolecular arrangements and positions in a cell, membrane proteins are generally classified into the following six types: (1) GPI (Glycosylphosphatidylinositol)-anchor; (2) lipid-anchor; (3) multi-pass; (4) peripheral; (5) single-pass type I; (6) single-pass type II (Glycosylphosphatidylinositol)-anchor; (2) lipid-anchor; (3) multi-

Prediction method

In this section, the detailed procedure of the integrated prediction method was described. The integrated method included three methods: BLAST/PSI-BLAST method, network-based method and shortest-distance method.

Suppose there are \( n \) proteins, say \( p_1, p_2, \ldots, p_n \), with known types in the training set \( S \). For one protein \( p_i \) in \( S \), its types were encoded into a vector

\[
F_i = (f_{i1}, f_{i2}, \ldots, f_{in})^T
\]

where

\[
f_{ik} = \begin{cases} 
1 & \text{If } p_i \text{ has the } k^{th} \text{ type} \\
0 & \text{Otherwise} 
\end{cases}
\]

**BLAST/PSI-BLAST method.** The basic local alignment search tool (BLAST), initiated by Altschul et al. [30], can be used to search for local similarity between two sequences. The idea underlying BLAST is to employ similarity measure optimized by background probabilities to directly approximate sequence alignments based on dynamic programming algorithm. This method can detect weak but more biologically significant sequence similarities. Gapped BLAST and PSI-BLAST (Positional Specific Iterated BLAST) [31] are the improved versions, improving on three major aspects: consuming-time of the extension, gapped alignment, and iterative search. Here, the BLAST/PSI-BLAST program (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/release/2.2.26/) was adopted to calculate the alignment score, denoted by \( S_0(p_1, p_2) \), of proteins \( p_1 \) and \( p_2 \). For an uncharacterized membrane protein \( p_q \), we searched a protein \( p_i \) in \( S \) whose \( S_0(p_q, p_i) = \max \{ S_0(p_q, p_j) : 1 \leq j \leq n \} \). Then the types of \( p_k \) were assigned to \( p_q \) as the predicted results. However, if the protein \( p_q \) has no homologous proteins in \( S \), this method cannot provide meaningful predicted results. In this case, the following two prediction methods make further predictions.

**Network-based method.** This method utilized the information of protein interactions in human retrieved from STRING [32] (http://string.embl.de/), which was a well-known database covering 5,214,234 proteins with their interactions in 1,133 organisms. These interactions consist of known and predicted protein interactions including direct (physical) and indirect (functional) associations derived from the following four sources: (1) Genomic context; (2) High-throughput experiments; (3) (Conserved) coexpression; (4) Previous knowledge. There was a confidence score for every pairwise interaction quantifying the likelihood of the interaction occurrence. Let us denote the confidence score for every pairwise interaction between two proteins \( p_1 \) and \( p_2 \) as \( S(p_1, p_2) \). Two proteins were regarded interactive in this study if the confidence score of the interaction between them was greater than zero. For an uncharacterized protein \( p_q \), the confidence scores of interactions between \( p_q \) and proteins in \( S \) were represented by

\[
W = (w_{1q}, w_{2q}, \ldots, w_{nq})^T
\]

where \( w_{fq} \) denoted the confidence score of the interaction between \( p_q \) and \( p_i \) in \( S \), i.e. \( w_{fq} = S(p_q, p_i) \). The probability of assigning the protein \( p_q \) the \( k^{th} \) type was calculated by

\[
p_{kq} = \frac{\text{Number of proteins in class } k \text{ that } p_q \text{ is assigned}}{\text{Total number of proteins in class } k}
\]
The greater the probability \( P(p_{q} = k) \) was, the more probably the query protein belonged to the \( k^{th} \) type. To handling multiple types, we took the top \( t \) highest probability types as the predicted results, where \( t \) was regarded as the smallest integer larger than or equal to the average number of types in the dataset. That is, if \( P(p_{q} = k_{1}) > P(p_{q} = k_{2}) > \cdots > P(p_{q} = k_{t}) \), the protein would be predicted to have \( k_{1} \), \( k_{2} \), \cdots , \( k_{t} \) type. This method was very similar to that in our previous work [33], which was used to predict protein functions. However, this method was not always effective. If there were no interactive proteins with \( p_{q} \) in \( S \), the outputs of equation (4) were all zeros. In this case, the last method described below would make the final prediction.

**Shortest-distance method.** As mentioned above, the higher the confidence score between two proteins, the stronger the interaction, and the more the two proteins shared identical or similar types. As shown in **Figure 2(A)**, proteins \( a \) and \( b \) were assumed to have stronger interaction with each other than any other pairs, followed by proteins \( b \) and \( c \). In this case, protein \( a \) would more likely have the same types as protein \( b \), and protein \( b \) would more likely have the same types as protein \( c \). Consequently, it could be inferred that proteins \( a \) and \( c \) would more probably share the same types, however they were not interacted directly with each other. In view of this, we constructed a weighted graph \( G \) in which nodes represent proteins and an edge between two nodes existed if and only if the confidence score of the interaction between them was greater than zero. In addition, the weight of the edge between nodes \( n_{1} \) and \( n_{2} \) was defined as

\[
 w(n_{1}, n_{2}) = 1000 - S_{i}(p_{1}, p_{2})
\]

where \( S_{i}(p_{1}, p_{2}) \) was the confidence score of the interaction between proteins \( p_{1} \) and \( p_{2} \) which are corresponding proteins of nodes \( n_{1} \) and \( n_{2} \). As shown in **Figure 2(B)**, proteins \( a \) and \( c \) were each other the shortest-distance neighbors except their direct neighbors. Therefore, the shortest-distances between proteins in \( G \) hold the clue to function relationship between them. This kind of weighted graph had been used in our previous work [34]. Therefore, even if two proteins had no direct interaction, it was possible to utilize the shortest distance in \( G \) to infer its types. By applying Dijkstra’s algorithm which is one of the most well-known shortest-distance algorithms, the shortest distance between any two proteins \( p_{1} \) and \( p_{2} \) was calculated, denoted by \( S_{d}(p_{1}, p_{2}) \) and termed as shortest-distance score. For an uncharacterized membrane protein \( p_{q} \), its types were predicted as the same as those of protein \( p_{k} \) in the training set \( S \) whose

\[
 S_{d}(p_{q}, p_{k}) = \min \{ S_{d}(p_{q}, p_{i}) : 1 \leq i \leq n \}.
\]

**Integrated method for predicting membrane protein multiple types.** The integrated method combined the BLAST/PSI-BLAST method, the network-based method and the shortest-distance method, as shown in **Figure 3**. For an uncharacterized protein \( p_{q} \), the prediction procedure by using the integrated method was as follows:

\[
P(p_{q} = k) = \sum_{j=1}^{n} f_{j} w_{n_{j}}, k = 1, 2, \cdots , 6 \tag{4}
\]
(I) If there was a homologous protein with $p_i$ in the training set $S$, i.e. $S(p_i, p_j) > 0$ for some $k$, only the BLAST/PSI-BLAST method was applied.

(II) If no homologous proteins but there was an interactive protein with $p_i$ in the training set $S$, i.e. $S(p_i, p_j) > 0$ for some $k$, only the network-based method was applied.

(III) If it was not the two cases above, the shortest-distance method was finally used.

**Metrics**

Among the three ways examining performances of classification algorithms: cross-validation test, leave-one-out test and independent test, the leave-one-out test is considered as the best [35,36], because the independent test requires additional datasets and the cross-validation test generates unstable results, while the leave-one-out test always provides the unique results for a given a dataset. Here, the leave-one-out test was adopted.

The commonly used measures are sensitivity, specificity, accuracy and matthew’s correlation coefficient in binary classification problems were not applicable to this study problem as one protein may simultaneously have more than one types. Deng et al. [37] used both concepts of Precision and Recall to measure performances of methods for multi-label classification problems. Assume $RF_i = \{r_{i1}, r_{i2}, \ldots, r_{in}\}$ as a label set of actual types for the $i$-th protein in the dataset, and $PF_i = \{p_{i1}, p_{i2}, \ldots, p_{im}\}$ as its predicted label set of types, the Precision and Recall were defined as:

$$
\begin{align*}
\text{Precision} &= \frac{\sum_{i=1}^{n} |RF_i \cap PF_i|}{\sum_{i=1}^{n} |PF_i|} \\
\text{Recall} &= \frac{\sum_{i=1}^{n} |RF_i \cap PF_i|}{\sum_{i=1}^{n} |RF_i|}
\end{align*}
$$

(6)

where $|A|$ denoted the number of elements in set $A$, and $n$ referred to the total number of predicted proteins. However, neither Precision nor Recall can be used alone for the evaluation. If predicted types $PF_i$ contained all the elements in actual types $RF_i$, Recall would be equal to 1. This is of no mean, because any one can tell all possible types of a membrane protein. On the other hand, if the predicted types $PF_i$ was a non-empty subset of the actual types $RF_i$, i.e. $PF_i \subseteq RF_i$, Precision would be equal to 1. To overcome these weaknesses, we presented a new evaluation indicator called **Accuracy** ($\text{Acc}$), which was defined as:

$$
\text{Acc} = \frac{1}{n} \sum_{i=1}^{n} \left( \frac{|RF_i \cap PF_i|}{|RF_i|} \times \frac{1}{1 + |PF_i - RF_i|} \right)
$$

(7)

The first part $\frac{|RF_i \cap PF_i|}{|RF_i|}$ on the right of the equation was approximately equivalent to Recall, while $\frac{1}{1 + |PF_i - RF_i|}$ was a penalty for $PF_i$ due to incorrect predictions. When $PF_i$ was identical to $RF_i$, the penalty was of no role. On the contrary, the more incorrect type the $PF_i$ included, the lower the $\text{Acc}$ was. Therefore, $\text{Acc}$ reflected both Precision and Recall.

**Results and Discussion**

**Choice of E-value in the BLAST/PSI-BLAST method**

It was plotted in Figure 4 the performances of the BLAST/PSI-BLAST method with different E-values. It was obviously observed from Figure 4(A) that the lower the threshold of E-value chose in the BLAST program, the higher the $\text{Acc}$ was, however the more the number of annotated proteins remained from Figure 4(B). The commonly expected goal of prediction is of course to reach high $\text{Acc}$ and simultaneously reduce the number of unannotated membrane proteins. However, this ideal objective is impossible to obtain because we are caught in the dilemma of choosing the cutoff of E-value. While lower E-values increased the statistical significance of alignment scores, it would be too strict and would accordingly lessen the number of proteins considered homologies. On the contrary, higher E-values may result in wrong homology, and thus reduce $\text{Acc}$. To balance $\text{Acc}$ and the number of unannotated proteins, we set E-value 0.01 in this study.
Performances of the individual methods and the integrated method

Three methods and the integrated method were all conducted on the three datasets S1, S2 and S3, respectively, evaluated by the leave-one-out test. Results were shown in Tables 1-3. It can be observed from Table 1 that the BLAST/PSI-BLAST method achieved the best performance with the highest Acc 94.71%, 91.15% and 85.02% on datasets S1, S2 and S3, respectively. However, 481, 529 and 620 proteins cannot be annotated because there were no homologous proteins in the corresponding datasets. The network-based method achieved the second highest Acc, i.e. 66.68%, 62.46%, 58.75% on the three datasets, respectively. Since no interactive proteins can be found in the corresponding datasets, there were 86, 38, 41 proteins unannotated. Although Acc was lower, the number of unannotated proteins was much less than those in the BLAST/PSI-BLAST method. In the shortest-distance method, the lowest Acc achieved (54.97%, 48.75%, 44.99% on the

Figure 3. The program flow diagram of the integrated method.
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three datasets, respectively). However, all proteins can be annotated. The shortest distance method was capable of annotating all proteins, although it was least effective.

The integrated method combined the three methods above. The detail contributions of the three single methods were shown in Table 2. It was obvious that the BLAST/PSI-BLAST method contributed the most, annotating 2,402, 1,552 and 849 proteins and achieved Acc of 94.71%, 91.15% and 85.02% on datasets S1, S2 and S3, respectively. The network-based method annotated 467 of the remaining 481 unannotated proteins in S1, 513 of the remaining 596 in S2, and 596 of the remaining 620 in S3, respectively, and obtained Acc of 53.10%, 53.51%, and 52.60% on the three datasets. The rest proteins in the three datasets were predicted by the shortest-distance method with Acc of 28.57%, 28.13%, and 18.75% on the three datasets. By combining the above predicted results for each dataset, the integrated method reached Acc of 87.65%, 81.39%, and 70.79% on S1, S2, and S3, respectively, which was listed in column 2 of Table 3. It was suggested that the integrated method could balance the prediction accuracy and the ratio of annotated proteins, benefiting from the high accuracy of BLAST/PSI-BLAST method and the wide range of the network-based method and shortest-distance method.

Membrane proteins may have multiple types. Statistical analysis were performed on the proteins having 1, 2, 3 types in each of the three datasets, respectively, with results shown in Figure 5. It can be seen that the prediction of 1 type proteins was the best, the 2 type ones was the second, and the 3 type ones was the least. For the 2 type proteins, nearly 50% could be correctly predicted or partly correctly predicted. It was indicated that the presented method could handle the multi-type membrane protein predictions.

Comparison with other methods
We compared our method to two other methods: the nearest neighbor algorithm (NNA) based on pseudo amino acid composition (PseAAC) and the real weighted combination (RWC). The procedure of these two methods was described in the following two paragraphs and their performances on datasets S1, S2 and S3 were in the fourth paragraph of this section.

NNA based on PseAAC. The concept of PseAAC was originally initiated by Chou to predict protein subcellular localization and membrane protein types [38]. The main idea of this method was to encode each protein sequence into a numeric vector containing information of sequence order effects. Here, the brief description of this method was as follows. Detail description

![Figure 4. The performance of the BLAST/PSI-BLAST method under various E-values.](doi:10.1371/journal.pone.0093553.g004)

**Table 1. Performances of the three single methods tested on the three datasets.**

| Dataset | BLAST/PSI-BLAST method | Network-based method | Shortest-distance method |
|---------|------------------------|----------------------|-------------------------|
|         | Acc        | NU*       | Acc      | NU   | Acc       | NU   |
| S1      | 94.71%    | 481       | 66.68%   | 86   | 54.97%    | 0    |
| S2      | 91.15%    | 529       | 62.46%   | 38   | 48.75%    | 0    |
| S3      | 85.02%    | 620       | 58.75%   | 41   | 44.99%    | 0    |

*NU represents the number of unannotated membrane proteins.

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about this method can be found in Chou’s work [38]. Without loss of generality, let $P_1, P_2, \cdots, P_L$ be a protein sequence with $L$ amino acid residues. Accordingly, a set of discrete correlation factors can be computed by

$$
\theta_j = \frac{1}{L-j+1} \sum_{i=j}^{L} \Theta(P_i, P_{i+j}) \quad j = 1, 2, \ldots, L < L
$$

where $\Theta(P_i, P_j)$ is defined by

$$
\Theta(P_i, P_j) = [F(P_i) - F(P_j)]^2
$$

$F(P_i)$ is a feature value of the amino acid $P_i$. In fact, it is converted from the 20 original feature values of 20 amino acids by

$$
F(P_i) = \frac{F_o(P_i) - \sum_{X \in \text{AAS}} \frac{F_o(X)}{20}}{\sum_{X \in \text{AAS}} \frac{[F_o(X) - \sum_{X \in \text{AAS}} \frac{F_o(X)}{20}]^2}{20}}
$$

where AAS is a set consisting of 20 amino acids. If the original feature values were known, $\theta_1, \theta_2, \ldots, \theta_1$ can be computed by equations (8), (9) and (10). Table 4 listed the original feature values of 20 amino acids for some physicochemical and biochemical properties of amino acids. Then, the PseAAC of a protein sequence can be encoded into a numeric vector:

$$
V = [v_1, v_2, \cdots, v_{20}, v_{21}, \cdots, v_{20 + \lambda}]^T
$$

where $v_i(1 \leq i \leq 20 + \lambda)$ can be computed by

$$
v_i = \begin{cases} 
\frac{f_i}{\sum_{k=1}^{20} f_k} & 1 \leq i \leq 20 \\
\frac{f_{i-1}}{\sum_{k=1}^{20} f_k} & 21 \leq i \leq 20 + \lambda
\end{cases}
$$

where $\varpi$ is the weight for the sequence order effects, $f_k(1 \leq k \leq 20)$ the occurrence frequency of the 20 amino acids in the protein sequence. In this study, we set $\varpi = 0.15, \lambda = 50$ and considered the following five physicochemical and biochemical properties of amino acids: (1) Codon diversity; (2) Electrostatic charge; (3) Molecular volume; (4) Polarity; (5) Secondary structure. Their original feature values of each amino acid were listed in Table 4, which were retrieved from [39,40,41]. Each of the five properties contributed $\lambda = 50$ components to contain the sequence order effects. Therefore, there are totally $20 + 50 \times 5 = 270$ components which can comprise a 270-D vector to represent a protein sequence. The same PseAAC scheme to encode protein sequences including same parameters had been applied in Wang et al.’s study [42]. Given an uncharacterized protein $p_q$ and the training set $S = \{p_i, i = 1, 2, \ldots, n\}$, where $n$ is the size of the training set, the PseAAC of these proteins can be easily obtained. The NNA based on PseAAC computed the distance between $p_q$ and $p_i(1 \leq i \leq n)$ by the following equation:

$$
D(p_q, p_i) = 1 - \frac{V(p_q) \cdot V(p_i)}{\|V(p_q)\| \cdot \|V(p_i)\|}
$$

where $V(P_i)$ is the PseAAC of protein $P_i$. The query protein $p_q$ was then assigned the types of protein $p_i$ in the training set whose

$$
D(p_q, p_i) = \min\{D(p_q, p_k) : 1 \leq i \leq n\}.
$$

RWC. RWC utilized another integrated way combining the information of BLAST alignment score, protein interaction confidence score and shortest-distance score. For two proteins $p_1$ and $p_2$, the score measuring their relationship was calculated by

$$
S_{\text{Integrated}}(p_1, p_2) = S_s(p_1, p_2) \times 0.8 + S_i(p_1, p_2) \times 0.15 + S_d(p_1, p_2) \times 0.05
$$

Given an uncharacterized membrane protein $p_q$, its types were predicted to be the types of protein $p_i$ in the training set whose

$$
S_{\text{Integrated}}(p_q, p_k) = \max\{S_{\text{Integrated}}(p_q, p_k) : 1 \leq i \leq n\}.
$$

Performance of NNA based on PseAAC and RWC. The NNA based on PseAAC and RWC were also conducted on the three datasets S1, S2 and S3, respectively, evaluated by leave-one-out test, with results listed in Table 3. The accuracy obtained by NNA based on PseAAC on S1, S2 and S3 was 70.41%, 61.70% and

Table 2. Contributions of the three methods to the integrated method.

| Dataset | BLAST/PSI-BLAST method | Network-based method | Shortest-distance method |
|---------|------------------------|----------------------|-------------------------|
|         | Acc  | NA* | Acc  | NA  | Acc  | NA  |
| S1      | 94.71% | 2,402 | 53.10% | 467 | 28.57% | 14 |
| S2      | 91.15% | 1,552 | 53.51% | 513 | 28.13% | 16 |
| S3      | 85.02% | 849  | 52.60% | 596 | 18.75% | 24 |

*NA represents the number of annotated membrane proteins.

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Table 3. Comparison of the integrated method with NNA based on PseACC and RWC on the three datasets.

| Dataset | The integrated method | NNA based on PseACC | RWC |
|---------|-----------------------|---------------------|-----|
| S1      | 87.65%                | 70.41%              | 81.34% |
| S2      | 81.39%                | 61.70%              | 71.40% |
| S3      | 70.79%                | 56.33%              | 56.82% |

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Table 4. Comparison of the integrated method with NNA based on PseACC and RWC on the three datasets.
Figure 5. Distributions of correct and incorrect predictions for different multi-type proteins. The subfigures A, B and C were the predicted results on the dataset S1, while D, E and F were for those on the dataset S2, and G, H and I for S3. c denotes the number of completely correct predictions of membrane proteins, e is the number of incorrect predicted membrane proteins, and p represents the number of partly correct type predictions.

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Table 4. Original feature values of physicochemical and biochemical properties of the 20 amino acids.

| Amino acid | Polarity | Second structure | Molecular volume | Codon diversity | Electrostatic charge |
|------------|----------|------------------|------------------|----------------|---------------------|
| A          | 0.591    | 1.302            | 0.733            | 1.57           | -0.146              |
| C          | -1.343   | 0.465            | -0.862           | -1.02          | -0.255              |
| D          | 1.05     | 0.302            | 3.656            | 0.259          | -3.242              |
| E          | 1.357    | -1.453           | 1.477            | 0.113          | -0.837              |
| F          | -1.006   | -0.59            | 1.891            | -0.397         | 0.412               |
| G          | -0.384   | 1.652            | 1.33             | 1.045          | 2.064               |
| H          | 0.336    | -0.417           | -1.673           | -1.474         | -0.078              |
| I          | -1.239   | -0.547           | 2.131            | 0.393          | 0.816               |
| K          | 1.831    | -0.561           | 0.533            | -0.277         | 1.648               |
| L          | -1.019   | -0.987           | -1.505           | 1.266          | -0.912              |
| M          | -0.663   | -1.524           | 2.219            | -1.005         | 1.212               |
| N          | 0.945    | 0.828            | 1.299            | -0.169         | 0.933               |
| P          | 0.189    | 2.081            | -1.628           | 0.421          | -1.392              |
| Q          | 0.931    | -0.179           | 3.005            | -0.503         | -1.853              |
| R          | 1.538    | -0.055           | 1.502            | 0.44           | 2.807               |
| S          | 0.228    | 1.399            | -4.76            | 0.67           | -2.647              |
| T          | -0.032   | 0.326            | 2.213            | 0.908          | 1.313               |
| V          | -1.337   | -0.279           | -0.544           | 1.242          | -1.262              |
| W          | -0.595   | 0.009            | 0.672            | -2.128         | -0.184              |
| Y          | 0.26     | 0.83             | 3.097            | -0.838         | 1.512               |

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membrane protein multi-types.

Discussions

Membrane proteins are embedded in or temporarily attached to the phospholipid bi-layer of the membrane. They interact with membranes via specific structures or conformations. Therefore, the protein structures were regarded as the determinate factors in the in vivo types. Based on the principle of Lock and Key Theory, the affinity and specificity of molecules is strictly determined by the local binding site shape and binding pocket size [43,44].

In this paper, we employed the sequence alignment rather than protein structures for the general concept that similar amino acid sequences were prone to have similar structures. Indeed, homology modeling predictions derived from sequence alignment methods were regarded as precise and ubiquitous for analyzing structures and types [55]. Therefore, the BLAST/PSI-BLAST was efficient to acquire the optimal prediction. Take the membrane protein ICAM5_HUMAN (uniprot: Q9UMF0) as an example. This protein belongs to single-pass type I. In the dataset S3, it was correctly predicted by the integrated method (via BLAST/PSI-BLAST method indeed) but wrongly by NNA based on PseAAC and RWC. The homologous proteins with Q9UMF0 in S3 and their types were shown in Figure 6(A). It was obvious that the protein shared the same or similar types with most of its homologous proteins (number 5 in the Figure 6(A)).

Interactive proteins could share the same or similar types since they have close distance when they perform biological functions in vivo. In Figure 6(B), shown were all the interactive proteins of another example membrane protein PEX12_HUMAN (uniprot: O00623) in the dataset S3, with their types. By our integrated method (via network-based method indeed), it was correctly predicted to be type of multi-pass, but wrongly to be type of peripheral by NNA based on PseAAC and to be type of single-pass single I by RWC. In the integrated method, the BLAST/PSI-BLAST can utilize the entire sequence order, and STRING can directly use the comprehensive functional-links. However, pseudo amino acid composition only can represent local information of sequence order. In RWC, the score calculated by equation (14) between membrane proteins was the weighted combination of alignment score, confidence score and shortest-distance score. The three types of scores were obtained in different ways; simple combination of them cannot characterize the complex in type relationships between membrane proteins. Experimental results also suggested that the combination of the three scores was less efficient than those by using separate ones.

However, the integrated method had some drawbacks because the BLAST/PSI-BLAST method was not primarily designed for specific proteins. Applying the “low-complexity filter” option in BLAST/ PSI-BLAST was not recommended for membrane proteins consisting of hydrophobic regions, because these regions could be the main factor determining the conformation and this would probably cause omission of the hydrophobic regions [56].

There was another problem that BLAST method would fail when membrane proteins have no homologies with known membrane proteins. However, it was still possible to discover the types according to the similarity of motifs or certain patterns with known proteins. Considering the variability of membrane proteins and the limited number of experimental high-resolution membrane protein structures, we did not further employ the similarity of motifs. This would probably reduce the prediction performance. Instead, the network-based method was implemented. Interaction of proteins might help and thus prediction would be more accurate. Although our prediction accuracy \( \text{Acc} = 87.65\%, 81.39\%, 70.79\% \) prima facie suggested our integrated method performed better than individual predictions (except the BLAST method which was the most reliable one), this method come with a caveat. As the BLAST/PSI-BLAST method was suitable to annotate 2,402 of 2,883, 1,552 of 2,081, and 849 of 1,469 proteins and obtained \( \text{Acc} \) of 94.71\%, 91.15\% and 85.02\%, respectively, it was quite possible that higher accuracy of the combined method was ascribed to the constitution of our dataset where most proteins have homologies and were annotated by the BLAST/PSI-BLAST method. However, in most cases, we were confronted with a...
dataset with few proteins having homologues with known proteins. Despite the most reliable predictions by BLAST, the network-based method was still required to annotate the remaining proteins. Here, we combined these different approaches to complement each other. Our integrated method benefited from proteins. Here, we combined these different approaches to based method was still required to annotate the remaining dataset with few proteins having homologies with known proteins. Despite the most reliable predictions by BLAST, the network-based method was still required to annotate the remaining proteins. Here, we combined these different approaches to complement each other. Our integrated method benefited from proteins. Here, we combined these different approaches to

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