Prediction of presynaptic and postsynaptic neurotoxins by combining various Chou’s pseudo components

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Presynaptic and postsynaptic neurotoxins are two groups of neurotoxins. Identification of presynaptic and postsynaptic neurotoxins is an important work for numerous newly found toxins. It is both costly and time consuming to determine these two neurotoxins by experimental methods. As a complement, using computational methods for predicting presynaptic and postsynaptic neurotoxins could provide some useful information in a timely manner. In this study, we described four algorithms for predicting presynaptic and postsynaptic neurotoxins from sequence driven features by using Increment of Diversity (ID), Multinomial Naive Bayes Classifier (MNBC), Random Forest (RF), and K-nearest Neighbours Classifier (IBK). Each protein sequence was encoded by pseudo amino acid (PseAA) compositions and three biological motif features, including MEME, Prosite and InterPro motif features. The Maximum Relevance Minimum Redundancy (MRMR) feature selection method was used to rank the PseAA compositions and the 50 top ranked features were selected to improve the prediction accuracy. The PseAA compositions and three kinds of biological motif features were combined and 12 different parameters that defined as P1-P12 were selected as the input parameters of ID, MNBC, RF, and IBK. The prediction results obtained in this study were significantly better than those of previously developed methods.

Neurotoxins can be divided into presynaptic and postsynaptic neurotoxins based on their mechanism of action1. Presynaptic neurotoxins are commonly called β-neurotoxins. These neurotoxins act on the plasmatic membranes of nerve endings, promote the generation of interterminal signals, and lead to a massive stimulation of the release of the neuromediator2–4. Presynaptic neurotoxins are rich sources of phospholipases5–9 and produce neuromuscular blockade by inhibiting the release of acetylcholine from the presynaptic membrane10. Postsynaptic neurotoxins are commonly called α-neurotoxins11–13, and most of these neurotoxins are from the venoms of snakes of families. Postsynaptic neurotoxins bind specially to the nicotinic acetylcholine receptor resulting in the prevention of nerve transmission, leading to death from asphyxiation14–17. Due to postsynaptic neurotoxins have similarity action to the reversible acetylcholine receptor antagonist curare with curare-mimetic toxins, there are often referred to as “curare-mimetic toxins”18. These two neurotoxins contribute to the understanding of the molecular steps of neurotransmission, and have potential use in cell biology and neuroscience research as well as therapeutics in some human neurological disorders. For example, presynaptic neurotoxins have been used for the treatment of migraine headache and cerebral palsy18. With the numerous of neurotoxin sequences generated in the post-genomic era, it is desired to develop a method for identification of neurotoxins for basic research and drug discovery.

In recent years, many computational algorithms have been developed for analyzing and predicting toxins. Short animal toxin and toxin-like protein sequences can be predicted by the web-based classifier ClanTox19, 20. The neurotoxins and bacterial toxins derived from Swiss-Prot were predicted by Feed-forwarded Neural Network

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(FNN), Partial Recurrent Neural Network (RNN) and Support Vector Machine (SVM)21–23. Four kinds of conotoxin superfamilies for 116 conotoxin sequences were predicted by ISort predictor, Least Hamming, Multi-class SVMs, one-versus-rest SVMs24, modified Mahalanobis discriminant25, and dHKNN26. Four conotoxin superfamilies for 261 conotoxin sequences that collected from Swiss-Prot were predicted by SVM27. In our previous work, based on the Animal Toxin Database (ATDB)28,29, the presynaptic and postsynaptic neurotoxins were predicted by Increment of Diversity (ID)30, and the correlation coefficient (CC) value was 0.7963 when evaluated by the jackknife test.

In this study, four algorithms were proposed for predicting presynaptic and postsynaptic neurotoxins by using Increment of Diversity (ID), Multinomial Naive Bayes Classifier (MNBC), Random Forest (RF), and K-nearest Neighbours Classifier (IBK). Pseudo amino acid (PseAA) compositions, MEME motif features31, Prosite motif features32 and InterPro motif features33 were used to represent the protein sequences. The Maximum Relevance Minimum Redundancy (MRMR)34,35 was used to rank the features for improving the performance of the predictors. When these algorithms were applied to the neurotoxin dataset with 78 presynaptic neurotoxins and 69 postsynaptic neurotoxins, the overall success rates obtained by the jackknife test were significantly higher than those of existing classifier on the same dataset. In addition, as demonstrated by a series of recent publications36–43 in compliance with Chou’s 5-step rule44, to establish a really useful sequence-based statistical predictor for a biological system, we should follow the following five guidelines: (a) construct or select a valid benchmark dataset to train and test the predictor; (b) formulate the biological sequence samples with an effective mathematical expression that can truly reflect their intrinsic correlation with the target to be predicted; (c) introduce or develop a powerful algorithm (or engine) to operate the prediction; (d) properly perform cross-validation tests to objectively evaluate the anticipated accuracy of the predictor; (e) establish a user-friendly web-server for the predictor that is accessible to the public. Below, we are to describe how to deal with these steps one-by-one.

**Results**

**Phylogenetic trees of presynaptic and postsynaptic neurotoxins.** In this study, the Molecular Evolutionary Genetics Analysis (MEGA) software45 was used to provide the phylogenetic trees of presynaptic and postsynaptic neurotoxins, only the neurotoxins that had the signal peptides were uploaded to the MEGA software for generating phylogenetic trees. The phylogenetic trees for presynaptic and postsynaptic neurotoxins were shown in Fig. 1A and B, respectively. These two figures illustrated some useful information about the inferred evolutionary relationships among those two neurotoxins, and the neurotoxins that in the same branch were believed to have a common ancestor. The Fig. 1A and B may also help us to better understand how the presynaptic and postsynaptic neurotoxins diversified over times.

**Analysis of Prosite motif features.** In 78 presynaptic neurotoxins, PS00118 was conserved in 29 sequences and PS00119 was conserved in 31 sequences. PS00118 is a pattern of phospholipase A2 histidine active site which is centered on the active site histidine and PS00119 is a pattern of phospholipase A2 aspartic acid active site which is centered on the active site aspartic acid. Both PS00118 and PS00119 contain three cysteines that involved in disulfide bonds. PS60004 belongs to PROSITE documentation PDOC60004 which is a pattern of omega-conotoxin family signature, and appears in 19 presynaptic neurotoxins. Omega conotoxins are calcium channel blockers and the cysteine arrangement [C-C-CC-C-C] is included in PS60004. PS00280, PS01138, PS01186, PS60015, PS60021, PS60022, PS60023 and PS60025 are also observed in presynaptic neurotoxins. PS00272 is a pattern of snake toxin signature and observed in 49 sequences. Snake toxins are a group of short and long neurotoxins, cytotoxins, short toxins and miscellaneous venom peptides. Snake toxin signature includes four conserved cysteines and a conserved proline is thought to be important for the maintenance of the tertiary structure. The second cysteine in this pattern is linked to the third cysteine by a disulfide bond. PS60014 is a pattern of...
alpha conotoxin family signature and appears in 8 postsynaptic neurotoxins. This pattern includes a common part of the cysteine arrangement [CC-C-C], four conserved cysteines are believed to be important for the maintenance of the tertiary structure of alpha conotoxins.

The comparison of MEME motifs (Fig. 2) with Prosite motifs shows that the conserved region from the fourth site to the eleventh site in the presynaptic neurotoxin motif 2 is corresponded to PS000118, this indicate that the presynaptic neurotoxin motif 2 may have the biological function of PS000118; PS000119 is corresponded to the conserved region from the third site to the eleventh site in the presynaptic neurotoxin motif 3; for PS00272, the conserved region from the tenth site to the twenty second site is corresponded to the first site to the twelfth site in the postsynaptic neurotoxin motif 2.

Prediction of presynaptic and postsynaptic neurotoxins. In order to investigate the influence of different parameters on the prediction quality, 12 different parameters were selected as the input parameters of ID, MNBC, RF, and IBK. The jackknife test results obtained by ID, MNBC, RF, and IBK with 12 different parameters were shown in Tables 1 and 2, Fig. 3A and B.

In this study, when using P12 as the input parameters of ID, MNBC, RF, and IBK for predicting presynaptic and postsynaptic neurotoxins, the overall accuracy of 95.92% and the CC value of 0.9208 were obtained by MNBC and RF, which were the highest overall accuracy and CC value in this study, and were also higher than the predictive results in our previous work.

For prediction of presynaptic and postsynaptic neurotoxins, based on the same input parameters, generally speaking, MNBC had the best prediction quality among four algorithms. For example, based on the parameters of P1, P2, P3, P4, P7, P8 and P12, the CC values were 0.8227, 0.8361, 0.8497, 0.8497 and 0.8635, respectively, which were higher than the CC value obtained by P1. Similarly, the higher CC values could also be obtained by MNBC, RF and IBK when using the same parameters. In addition, we found that the predictive results obtained by 19 motifs (13 Prosite motifs and 6 MEME motifs) were better than those obtained by 13 Prosite motifs or 6 MEME motifs in most cases. These results clearly illustrated that MEME motifs, Prosite motifs and InterPro motifs could significantly improve the predictive power of ID, MNBC, RF and IBK for predicting the presynaptic and postsynaptic neurotoxins.

Based on the same algorithm, it was clear that the performances were improved when sequence derived features and motif features were used as input parameters, when compared with other sequence derived features. For ID, when using P2, P3, P4, P5 and P6 as the input parameters, the CC values were 0.8361, 0.8227, 0.8497, 0.8497 and 0.8635, respectively, which were higher than the CC value obtained by P1. Similarly, the higher CC values could also be obtained by MNBC, RF and IBK when using the same parameters. In addition, we found that the predictive results obtained by 19 motifs (13 Prosite motifs and 6 MEME motifs) were better than those obtained by 13 Prosite motifs or 6 MEME motifs in most cases. These results clearly illustrated that the MEME motifs, Prosite motifs and InterPro motifs could significantly improve the predictive power of ID, MNBC, RF and IBK for predicting the presynaptic and postsynaptic neurotoxins.

In this study, the prediction performance was improved by the effective feature selection method when using the same algorithm. Tables 1 and 2 illustrated that the results of the ID, MNBC, RF and IBK with the parameters of P1-P7. Except for the predictive results of IBK, it was clear that higher or equivalent overall accuracy had been obtained by the proposed algorithms with the parameter of P7, when compared with the overall accuracy obtained by the parameters of P1-P6. For example, for the problem of presynaptic and postsynaptic neurotoxins prediction, when P7 was selected as the input parameter, the CC value was 0.8786 for ID, which was 0.0823, 0.0425, 0.0559, 0.0289, 0.0289, and 0.0151 higher than those of P1-P6, respectively. Similarly, except for the predictive results of IBK, the CC value obtained by P7 for MNBC, and RF were also higher than those of P1-P6. These results clearly indicated that MMRM feature selection method was effective and helpful for the prediction of presynaptic and postsynaptic neurotoxins.

For the problem of presynaptic and postsynaptic neurotoxins prediction, as shown in Tables 1 and 2, the sensitivity of presynaptic neurotoxins and the specificity of postsynaptic neurotoxins varied significantly with the
parameters, indicating that the prediction results of presynaptic neurotoxins were more correlated with different parameters than the prediction results of postsynaptic neurotoxins. That was because more protein motifs were discovered in the presynaptic neurotoxins than in the postsynaptic neurotoxins. For example, 11 Prosite motifs were discovered by ScanProsite in the presynaptic neurotoxins, however, only 2 Prosite motifs were discovered by ScanProsite in the postsynaptic neurotoxins.

As shown Tables 1 and 2, the best predictive results of ID were obtained by using P10 as the input parameter. In this case, all of the presynaptic neurotoxins were predicted correctly, and 7 postsynaptic neurotoxins were predicted incorrectly. The Animal Toxin database entries numbers of these 7 postsynaptic neurotoxins were AT0001110, AT0000526, AT0002477, AT0000527, AT0000327, AT0002380 and AT0000334, respectively. MEME motifs were not discovered in these postsynaptic neurotoxins, only Prosite motifs and InteroPro motifs were discovered in AT000110 and AT0002380. However, AT000110 and AT0002380 not only belonged to the presynaptic neurotoxins but also belonged to the postsynaptic neurotoxins, and in this case, they were predicted as the presynaptic neurotoxins. Based on these results, we suspected that the motif features may provide an important role in the problem of presynaptic and postsynaptic neurotoxins prediction.

**Discussion**

In this paper, in order to predict presynaptic and postsynaptic neurotoxins, 12 different parameters were selected as the input parameters of ID, MNBC, RF, and IBK. The prediction results of the jackknife test were shown in Tables 1 and 2, and Fig. 3. Based on the similar results of different methods presented in Tables 1 and 2, and Fig. 3, we suspected that when using the same parameters, ID, MNBC, RF, and IBK had little impact on prediction results for predicting presynaptic and postsynaptic neurotoxins, and this may be an intrinsic characteristics of...
machine learning algorithms which also occurred in the other prediction problems. However, we also found that
the input parameters have big impact on prediction results. Taking the ID algorithm as an example, we found
that the Acc can increase from 89.80% to 95.24%, and the CC can increase from 0.7963 to 0.9080 for prediction
the presynaptic and postsynaptic neurotoxins. Similar improved Acc and CC can also be obtained by other three
algorithms. So, the input parameters should have more impact on the prediction results.

In our previous work\(^3\), for using the same dataset, 78 presynaptic neurotoxins and 69 postsynaptic neurotox-
ins were predicted by Increment of Diversity (ID), the highest Sn, Sp and CC obtained in our previous work were
88.46%, 92.00% and 0.7963 for presynaptic neurotoxins, and were 91.30%, 87.50% and 0.7963 for postsynaptic
neurotoxins, respectively. In this study, we found that, the best Sn, Sp and CC were 100.0%, 92.86% and 0.9208 for
presynaptic neurotoxins, and were 91.30%, 100.0%, and 0.9208 for postsynaptic neurotoxins, respectively. Based
on these results, we can conclude that the prediction algorithms presented in this study had some advantage over
the previous one.

With the increased number of toxins in the public dataset, it is indispensable to develop some reliable meth-
ods for classification of presynaptic and postsynaptic neurotoxins. In this study, ID, MNBC, RF, and IBK were
applied to classify presynaptic and postsynaptic neurotoxins, a new promising feature representation method was
presented by embedding PseAA compositions, MEME motif features, Prosite motif features and InterPro motif
features to represent a protein sample. The MRMR feature selection method was also used to select 50 top ranked
PseAA compositions to improve the predictive results. In order to obtain the best performance of the proposed
algorithms, different kinds of motif features and PseAA compositions were combined and selected as the input
parameters of four algorithms. The predictive results presented in this study clearly indicated: (1) MRMR feature
selected method, complemented with motif features can significantly improve the prediction quality of neuro-
toxins; (2) using different parameters would make it possible for algorithms to perform better than the others.
The best prediction results were obtained when using 50 PseAA compositions, 46 InterPro motif features and 6
MEME motif features as the input parameters of MNBC and RF. In summary, the above results indicated that
ID, MNBC, RF and IBK by using 50 PseAA compositions and biological motif features as the input parameters
were reliable for prediction of presynaptic and postsynaptic neurotoxins. We hope that the machine learning
algorithms will provide some support for the identification of neurotoxins in the future. The proposed algorithms
may become the useful tools in bridging the gap between the huge number of toxins in the public databases and
the relatively less number of toxins that have been functionally characterized. As pointed out in Shen and Chou\(^4\) and
demonstrated in a series of recent publications\(^6, 7, 10, 46–54\), user-friendly and publicly accessible web-servers
represent the future direction for developing practically more useful methods that will significantly enhance their
impacts\(^5\), we shall make efforts in our future work to provide a web-server for the analysis method presented in
this paper.
Methods

Datasets. The dataset generated by Yang and Li was used to estimate the effectiveness of the new prediction methods. The protein sequences in this dataset were downloaded from the Animal Toxin Database (ATDB). The PISCES was used to cull the presynaptic and postsynaptic neurotoxin sequences where no two proteins in each dataset had more than 80% sequence identity. In the final dataset, presynaptic neurotoxin dataset consists of 78 protein sequences, and postsynaptic neurotoxin dataset consists of 69 protein sequences.

Machine learning approaches. In this study, Increment of Diversity (ID), Multinomial Naive Bayes Classifier (MNBC), Random Forest (RF), and K-nearest Neighbours Classifier (IBK) were used to classify the presynaptic and postsynaptic neurotoxins. The ID algorithm was implemented in the C++ software while the rest of the algorithms were implemented in the Weka package.

Pseudo amino acid composition. It is very important to select a set of reasonable parameters for protein sequences prediction. As mentioned in previous works, pseudo amino acid composition (PseAAC) is a widely used approach for representation of protein sequences, and can be generated by a series powerful web-servers developed recently. In this study, according to the concept of the Chou’s PseAA compositions, 400 dipeptide compositions were selected as the parameters of our approaches, which were defined in 400-dimension (400-D) space, formulated as:

\[ Y: \{ y_1, y_2, \ldots, y_{400} \} \]  

where \( y_i \) (i = 1, 2, 3 … 400) was the absolute occurrence frequencies of 400 dipeptides.

Maximum Relevance Minimum Redundancy. In this study, MRMR was applied on 400 PseAA compositions. After considering both the predictive accuracy and the MRMR score, the top 50 features were selected as the input parameters of the machine learning algorithms, which were defined in a 50-dimension (50-D) space, formulated as:

\[ Z: \{ z_1, z_2, z_3, \ldots, z_{50} \} \]

MEME motif features. In this study, the presynaptic and postsynaptic neurotoxin datasets were uploaded to MEME software to conduct motif search. The maximum motif number was set to 3 and the maximum motif length was set to 15. The logo format and the regular expression of these motifs were shown in Fig. 2. Six MEME motifs had been created which were corresponded to the presynaptic neurotoxins and postsynaptic neurotoxins, and the number of motif features was 6. Each element of the vectors represented the presence or absence of a motif in the protein sequences. That was, the corresponded feature value was 1 if a motif was presented; otherwise, it was 0. Consequently, each protein sequence was converted into a 6-dimension (6-D) space, formulated as:

\[ M: \{ m_1, m_2, \ldots, m_6 \} \]

Prosite motif features. In this study, 11 kinds of Prosite motifs were found in 78 presynaptic neurotoxin sequences and 2 kinds of Prosite motifs were found in 69 postsynaptic neurotoxin sequences. The total number of motif features was 13. Consequently, each protein sequence was converted into a 13-dimension (13-D) space, formulated as:

\[ P: \{ p_1, p_2, \ldots, p_{13} \} \]

InterPro motif features. InterPro is an integrated database of protein families, domains and functional sites. In this study, 78 presynaptic neurotoxin sequences and 69 postsynaptic neurotoxin sequences were scanned by InterPro, and 46 functional motifs were found in the neurotoxin datasets. The total number of motif features was 46. Consequently, each protein sequence was converted into a 46-dimension (46-D) space, formulated as:

\[ N: \{ n_1, n_2, \ldots, n_{46} \} \]

Features for prediction algorithms. In order to improve the prediction accuracy, 400 PseAA compositions, 50 PseAA compositions, 13 kinds of Prosite motifs, 6 kinds of MEME motifs and 46 InterPro motifs were combined. Because the Prosite motifs were contained in the InterPro motifs, so 13 Prosite motifs were not combined with 46 InterPro motifs. P1-P12 indicated 12 kinds of parameters, and these parameters were selected as the input parameters of ID, MNBC, RF, and IBK (Table 3).

Evaluation of methods. In this study, in order to roundly estimate the accuracy of our predictor, the sensitivity, specificity, correlation coefficient and overall accuracy were also calculated:
where TP denoted the numbers of the correctly recognized positives, FN denoted the number of the positives recognized as negatives, FP denoted the number of the negatives recognized as positives, TN denoted the numbers of correctly recognized negatives, N was the total number of protein sequences.

The set of metrics is valid only for the single-label systems. For the multi-label systems whose existence has become more frequent in system biology and system medicine, a completely different set of metrics as defined in work of Chou is needed. In order to take the advantage of using the Chou’s intuitive set of metrics for studying protein signal peptide cleavage site, the TP, TN, FP, and FN can be represented as follows:

\[
\begin{align*}
\text{Sn} &= \frac{TP}{TP + FN} \\
\text{Sp} &= \frac{TP}{TP + FP} \\
\text{CC} &= \frac{(TP \times TN) - (FP \times FN)}{\sqrt{(TP + FP) \times (TN + FN) \times (TP + FN) \times (TN + FP)}} \\
\text{Acc} &= \sum \frac{TP_i}{N}
\end{align*}
\]

(6)

Substituting Eq. (7) into Eq. (6), we can obtain the following metrics:

\[
\begin{align*}
\text{Sn} &= 1 - \frac{N^+}{N} \\
\text{Sp} &= \frac{N^+ - N^-}{N^+ - N^- + N^+} \\
\text{Acc} &= 1 - \frac{N^+ + N^-}{N^+ + N^-} \\
\text{CC} &= \sqrt{\frac{1 + \frac{N^- - N^+}{N^+}}{1 + \frac{N^+ - N^-}{N^-}}}
\end{align*}
\]

(8)

where \(N^+\) denoted the total numbers of the positives, \(N^-\) denoted the total numbers of the negatives, \(N^-\) denoted the number of the negatives incorrectly predicted as positives, and \(N^+\) denoted the number of the positives incorrectly predicted as negatives. In addition, the jackknife test was also used to validate the prediction power of our algorithms.
References

1. Afifiyan, F. et al. Four new postsynaptic neurotoxins from Naja naja sputatrix venom: cDNA cloning, protein expression, and phylogenetic analysis. *Toxicol. Lett.* 149, 91–101 (2004).

2. Harris, J. B. Polyepitopes from snake venom which act on nerve and muscle. *Prog. Med. Chem.* 21, 63–110 (1984).

3. Afifiyan, F., Armugam, A., Tan, C. H., Gopalakrishnakone, P. & Jeyaseelan, K. Postsynaptic alpha-neurotoxin gene of the spitting cobra, Naja naja: structure, organization, and phylogenetic analysis. *Gene* 160, 259–266 (1999).

4. Gong, N., Armugam, A. & Jeyaseelan, K. Postsynaptic short-chain neurotoxins from Pseudonaja textilis. cDNA cloning, expression and protein characterization. *J. Biochem.* 165, 982–989 (1999).

5. Tamiya, T., Ohno, S., Nishimura, E., Fujimi, T. & Tsuichaya, T. Complete nucleotide sequences of cDNAs encoding long chain alpha-neurotoxins from sea krait, Laticauda semifasciata. *Toxicon* 37, 181–185 (1999).

6. Rossetto, O. & Montecucco, C. Presynaptic neurotoxins with enzymatic activities. *Handb. Exp. Pharmacol.* 129–170 (2008).

7. Naamati, G., Askenazi, M. & Linial, M. ClanTox: a classifier of short animal toxins. *Nucleic Acids Res.* 33, W363–W368 (2009).

8. Naamati, G., Askenazi, M. & Linial, M. A predictor for toxin-like proteins exposes cell modulator candidates within viral genomes. *Bioinformatics* 26, 1482–1488 (2010).

9. Guan, X. M., Guo, Y. Z., Wang, X. & Li, M. Prediction of neurotoxins by support vector machine based on multiple feature vectors. *Int. J. Mol. Sci.* 12, 241–246 (2010).

10. Saha, S. & Raghava, G. P. Prediction of neurotoxins based on their function and source. *In Silico Biol.* 6, 369–387 (2007a).

11. Saha, S. & Raghava, G. P. Predicting function and toxicity of bacterial toxins. *In Silico Biol.* 6, 405–412 (2007b).

12. Mondal, S., Bhavna, R., Mohan Babu, R. & Ramakumar, S. Pseudo amino acid composition and multi-class support vector machines approach for conotoxin superfamily classification. *J. Theor. Biol.* 243, 252–260 (2006).

13. Yin, J. B., Fan, Y. X. & Shen, H. B. Conotoxin superfamily prediction using diffusion maps dimensionality reduction and subspace classifier. *Curr. Protein Pept. Sci.* 12, 880–888 (2011).

14. Fan, Y. X., Song, J., Shen, H. B. & Kong, X. PredCSF: an integrated feature-based approach for predicting conotoxin superfamily. *Protein Pept. Lett.* 18, 261–267 (2011).

15. He, Q. et al. ATDB 2.0: A database integrated toxin-ion channel interaction data. *Toxicol.* 56, 644–647 (2010).

16. He, Q. Y. et al. ATDB: a uni-database platform for animal toxins. *Nucleic Acids Res.* 36, D293–D297 (2008).

17. Yang, L. & Li, Q. Prediction of presynaptic and postsynaptic neurotoxins by the increment of diversity. *Toxicol. In Vitro* 20, 346–348 (2009).

18. Bailey, T. L., Williams, N., Musleh, C. & Li, W. W. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* 34, W369–W373 (2006).

19. Sigrist, C. J. et al. PROSITE, a protein domain database for functional characterization and annotation. *Nucleic Acids Res.* 38, D161–D166 (2010).

20. Hunter, S. et al. InterPro: the integrative protein signature database. *Nucleic Acids Res.* 37, D211–D215 (2009).

21. Ding, C. & Peng, H. Minimum redundancy feature selection from microarray gene expression data. *J. Bioinform. Comput. Biol.* 3, 185–205 (2005).

22. Peng, H., Long, F. & Ding, C. Feature selection based on mutual information: criteria of max-dependency, max-relevance, and min-redundancy. *IEEE Trans. Pattern Anal. Mach. Intell.* 27, 1226–1238 (2005).

23. Liu, L. et al. pKNAm-PC: Predicting N6-methyladenosine sites in RNA sequences via physical–chemical properties. *Anal. Biochem.* 497, 60–67 (2016).

24. Chen, W., Tang, H., Ye, J., Lin, H. & Chou, K. C. iRNA-PseU: Identifying RNA pseudouridine sites. *Mol. Ther. Nucleic Acids* 5, e332 (2016).

25. Liu, L., Liu, Z., Xiao, X., Liu, B. X. & Chou, K. C. p5uc-Lyc: Predict lyso sine succinylation sites in proteins with PseAAC and ensemble random forest approach. *J. Theor. Biol.* 394, 223–230 (2016).

26. Liu, B., Long, R. & Chou, K. C. iDHS-EL: Identifying DNase I hypersensitive sites by fusing three different modes of pseudo nucleotide composition into an ensemble learning framework. *Bioinformatics* 32, 2411–2418 (2016).

27. Cheng, X., Zhao, S. G., Xiao, X. & Chou, K. C. iATC-miSF: A multi-label classifier for predicting the classes of anatomical therapeutic chemicals. *Bioinformatics* 33, 341–346 (2017).

28. Chen, W. et al. RNA-AL: identifying the adenosine to inosine editing sites in RNA sequences. *Nucleic Acids Res.* 40, 8208–8217 (2017).

29. Meher, P. K., Sahu, T. K., Saini, V. & Rao, A. R. Predicting antimicrobial peptides with improved accuracy by incorporating the compositional, physico-chemical and structural features into Chou's general PseAAC. *Sci. Rep.* 7, 42362 (2017).

30. Liu, B., Wang, S. Y., Long, R. & Chou, K. C. iRSpot-EL: identify recombination spots with an ensemble learning approach. *Bioinformatics* 33, 35–41 (2017).

31. Chou, K. C. Some remarks on protein attribute prediction and pseudo amino acid composition. *J. Theor. Biol.* 273, 236–247 (2011).

32. Tamura, K., Dudley, J., Nei, M. & Kumar, S. MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24, 1596–1599 (2007).

33. Chou, K. C. & Shen, H. B. Rw: Recent advances in developing web-servers for predicting protein attributes. *Nat. Sci.* 1, 63–92 (2009).
Author Contributions

Y.L. and Y.Z. conceived and designed the experiments. H.H. and L.Y. performed the experiments. L.Y. and H.H. analyzed the data. S.W., and Y.L. contributed materials/analysis tools. H.H. and L.Y. wrote the paper.

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Chen, W., Ding, H. F. P. M., Lin, H. & Chou, K. C. iACP: a sequence-based tool for identifying anticancer peptides. Oncotarget 7, 16895–16909 (2016).

Jia, J. H., Zhang, I. X., Liu, Z., Xiao, X. & Chou, K. C. psu-mCD: predicting sumoylation sites in proteins with covariance discriminant algorithm by incorporating sequence-coupled effects into general PseAAC. Bioinformatics 32, 3133–3141 (2016).

Zhang, C. J. et al. iOr-Human: identify human origin of replication by incorporating dinucleotide physicochemical properties into pseudo nucleotide composition. Oncotarget 7, 69783–69793 (2016).

Jia, J. H., Liu, Z., Xiao, X., Liu, B. X. & Chou, K. C. iCar-PseCp: identify carbonylation sites in proteins by Monte Carlo sampling and incorporating sequence-coupled effects into general PseAAC. Oncotarget 7, 34558–34570 (2016).

Qu, W. R., Sun, B. Q., Xiao, X., Xu, Z. C. & Chou, K. C. iHyd-PseCp: Identify hydroxyproline and hydroxylysine in proteins by incorporating sequence-coupled effects into general PseAAC. Oncotarget 7, 44310–44321 (2016).

Qu, W. R., Xiao, X., Xu, Z. C. & Chou, K. C. iPhos-PseEn: Identifying phosphorylation sites in proteins by fusing different pseudo components into an ensemble classifier. Oncotarget 7, 51270–51283 (2016).

Xiao, X., Ye, H. X., Liu, Z., Jia, J. H. & Chou, K. C. iROS-gPseKNC: Predicting replication origin sites in DNA by incorporating dinucleotide position-specific propensity into general pseudo nucleotide composition. Oncotarget 7, 34180–34189 (2016).

Liu, B., Wu, H., Zhang, D. Y., Wang, X. L. & Chou, K. C. Pse-Analysis: a python package for DNA/RNA and protein/peptide sequence analysis based on pseudo components and kernel methods. Oncotarget 8, 13338–13343 (2017).

Chou, K. C. Impacts of bioinformatics to medicinal chemistry. Med. Chem. 11, 218–234 (2015).

Wang, G. & Dunbrack, R. L. Jr. PISCES: recent improvements to a PDB sequence culling server. Nucleic Acids Res. 33, W94–W98 (2005).

Wang, G. & Dunbrack, R. L. Jr. PISCES: a protein sequence culling server. Bioinformatics 19, 1589–1591 (2003).

Zhang, L. & Luo, L. Splice site prediction with quadratic discriminant analysis using diversity measure. Nucleic Acids Res. 31, 6214–6220 (2003).

Frank, E., Hall, M., Trigg, L., Holmes, G. & Witten, I. H. Data mining in bioinformatics using Weka. Bioinformatics 20, 2479–2481 (2004).

Chou, K. C. Prediction of protein cellular attributes using pseudo-amino acid composition. Proteins: Struct. Funct. Genet. 43, 246–259 (2001).

Chou, K. C. Using amphiphilic pseudo amino acid composition to predict enzyme subfamily classes. Bioinformatics 21, 10–19 (2005).

Du, P. F., Gu, S. W. & Jiao, Y. S. PseAAC-General: Fast Building Various Modes of General Form of Chou's Pseudo-Amino Acid Alphabet Composition for Large-Scale Protein Datasets. Int. J. Mol. Sci. 15, 3495–3506 (2014).

Liu, B. et al. Pse-in-One: a web server for generating various modes of pseudo components of DNA, RNA, and protein sequences. Nucleic Acids Res. 43, W65–W71 (2015).

Nanni, L., Brahnam, S. & Lumini, A. Prediction of protein structure classes by incorporating different protein descriptors into general Chou's pseudo amino acid composition. J. Theor. Biol. 360, 109–116 (2014).

Sharma, R. et al. Predict Gram-Positive and Gram-Negative Subcellular Localization via Incorporating Evolutionary Information and Physicochemical Features Into Chou's General PseAAC. IEEE T. Nanobiosci. 14, 915–926 (2015).

Tahir, M. & Hayat, M. iNuc-STNC: a sequence-based predictor for identification of nucleosome positioning in genomes by extending the concept of SAAC and Chou's PseAAC. Mol. Biosyst. 12, 2587–2593 (2016).

Rahimi, M., Bakhtiariadezh, M. R. & Mohammadi-Sangcheshmeh, A. Ooogenesis_Pred: A sequence-based method for predicting oogenesis proteins by six different modes of Chou's pseudo amino acid composition. J. Theor. Biol. 414, 128–136 (2017).

Chou, K. C. Pseudo amino acid composition and its applications in bioinformatics, proteomics and system biology. Curr. Proteomics 6, 262–274 (2009).

Zuo, Y. C. et al. PseKRAAC: a flexible web server for generating pseudo K-tuple reduced amino acids composition. Bioinformatics 33, 122–124 (2017).

Zuo, Y. C. et al. iDPF-PseRAAAC: A Web-Server for Identifying the Defensin Peptide Family and Subfamily Using Pseudo Reduced Amino Acid Alphabet Composition. PLoS One 10, e0145541 (2016).

Liu, B., Wu, H. & Chou, K. C. Pse-in-One 2.0: An Improved Package of Web Servers for Generating Various Modes of Pseudo Components of DNA, RNA, and Protein Sequences. Natural Science 09, 67–91 (2017).

Chou, K. C. & Cai, Y. D. Using functional domain composition and support vector machines for protein subcellular location. J. Biol. Chem. 277, 45765–45769 (2002).

Zuo, Y. C. et al. Discrimination of membrane transporter protein types using K-nearest neighbor method derived from the similarity distance of total diversity measure. Mol. Biosyst. 11, 950–957 (2015).

Zuo, Y. C. et al. Predicting peroxidase subcellular localization by hybridizing different descriptors of Chou’s pseudo amino acid patterns. Anal. Biochem. 458, 14–19 (2014).

Chou, K. C., Wu, Z. C. & Xiao, X. iLoc-Euk: A Multi-Label Classifier for Predicting the Subcellular Localization of Singleplex and Multiplex Eukaryotic Proteins. PLoS One 6, e18258 (2011).

Qu, W. R., Sun, B. Q., Xiao, X., Xu, Z. C. & Chou, K. C. iPTM-mLys: identifying multiple lysine PTM sites and their different types. Bioinformatics 32, 3116–3126 (2016).

Chou, K. C. Some remarks on predicting multi-label attributes in molecular biosystems. Mol. Biosyst. 9, 1092–1103 (2013).

Chou, K. C. Prediction of protein signal sequences. Curr. Protein Pept. Sci. 3, 615–622 (2002).

Chen, W., Feng, P. M., Lin, H. & Chou, K. C. iSpot-PseDNC: identify recombination spots with pseudo dinucleotide composition. Mol. Biosyst. 41, e68–e68 (2013).

Chen, J. J., Long, R., Wang, X. L., Liu, B. & Chou, K. C. dHP-PseRA: detecting remote homology proteins using profile-based pseudo protein sequence and rank aggregation. Sci. Rep. 6, 3233 (2016).

Chen, W., Feng, P. M., Ding, H., Lin, H. & Chou, K. C. Using deformation energy to analyze nucleosome positioning in genomes. Genomics 107, 69–75 (2016).

Liu, B., Fang, L. Y., Long, R., Lan, X. & Chou, K. C. iEnhancer-2L: a two-layer predictor for identifying enhancers and their strength by pseudo k-tuple nucleotide composition. Bioinformatics 32, 362–369 (2016).

Author Contributions

L.Y., T.L., and Y.Z. conceived and designed the experiments. H.H. and L.Y. performed the experiments. L.Y. and H.H. analyzed the data. S.W., and Y.L. contributed materials/analysis tools. H.H. and L.Y. wrote the paper.
Additional Information

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