Artificial Intelligence in Prediction of Secondary Protein Structure Using CB513 Database

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Abstract

In this paper we describe CB513 a non-redundant dataset, suitable for development of algorithms for prediction of secondary protein structure. A program was made in Borland Delphi for transforming data from our dataset to make it suitable for learning of neural network for prediction of secondary protein structure implemented in MATLAB Neural-Network Toolbox. Learning (training and testing) of neural network is researched with different sizes of windows, different number of neurons in the hidden layer and different number of training epochs, while using dataset CB513.

1. PROTEIN STRUCTURE PREDICTION PROBLEM

1. 1. Introduction

Current technology for three-dimensional protein structure prediction relies on using unknown sequences of proteins and overlaying them with homologous proteins with known structure. Such practice is faulty as it does not account for proteins which do not have a homologous protein in their database or current technology is not yet able to identify existing homologous proteins. Most of today's algorithms which are employed in secondary protein structure prediction rely heavily on known three-dimensional structures. Using those algorithms certain parameters are then imposed upon unknown sequences. Such methods rely on available data for their predictions. Earlier algorithms for prediction of secondary protein structures reported high success, but these studies were based on small quantities of data which was mainly derived during training sessions. For example, Lim [1] claimed 70% Q3 prediction success of 25 proteins; Garnier [2] achieved 63% success for 26 proteins; while Qian and Sejnowski [3] reported 64.3% prediction success in 19 proteins. Using such a different protein pool for training and testing of algorithms produces a challenge for objective evaluation of such algorithms. Rost and Sander [4] tested a method in prediction of proteins in which they did not use the same proteins for the formation of their algorithm. They achieved prediction success better than 70%. Prediction success of Lim's study [1] was reduced by 14% down to 56%. Cross-validation of methods of testing whereby initial proteins are removed from the training pool of proteins yields more realistic predictions.

1.2 Problem with objective testing of methods in prediction of secondary protein structure

For a protein sequence with an unknown 3D structure which is similar to a protein with a known 3D structure, the best prediction model for protein's secondary structure is the alignment of sequences using algorithms derived from dynamic programming [5]. Methods for predicting secondary structures are employed when similar sequences are not available. Testing of exactness of a prediction on a training dataset yields unrealistically high success rates. An ideal approach is to test a desired sequence of a protein whose sequence is not included in the testing set and has no similarities.

Today there are around 500 different proteins with non-similar sequences with known 3D structures which can be used in assessing the accuracy of technology for prediction of secondary structure of proteins. However, many of today's prediction models are based on a set of 126 protein chains used by Rost and Sander [6]. Cuff J A and Barton G J developed a new non-redundant set of 396 protein domains which include proteins from RS126 protein chains [7]. In our research we used developed algorithm [8] using CB513 data set [7] and expanded application interface API-EPE2 with better performances in comparing to API-EPE [8].

2. EXTRACTION, PREPARING, AND ENCODING OF DATA SAMPLES

The concept of algorithm for predicting secondary protein structure is shown in Fig. 1. The implementation of this algorithm is provided with two software packages. The first one is **API_EPE**2 (**AP**plication **I**nterface used for **E**xtracting, **P**reparing and **E**ncoding), and second is MATLAB&NN Toolbox.

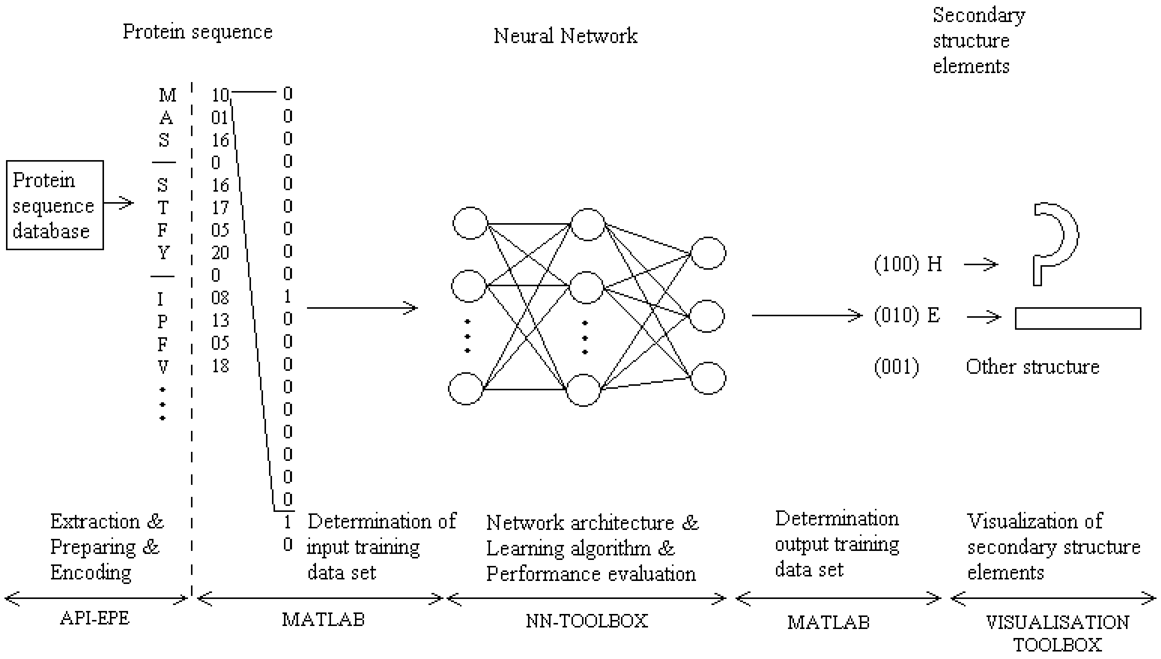

Protein sequence
Neural Network
Secondary structure elements
Protein sequence database
M A S — S T F Y — I P F V
10 01 16 0 16 17 05 20 0 08 13 05 18
0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 1 0
(100) H
(010) E
(001) Other structure
Extraction & Preparing & Encoding
Determination of input training data set
Network architecture & Learning algorithm & Performance evaluation
Determination output training data set
Visualization of secondary structure elements
API-EPE
MATLAB
NN-TOOLBOX
MATLAB
VISUALISATION TOOLBOX

Figure 1 Neural network can learn general rules of association between primary sequence of protein chains and corresponding secondary structures elements

The purpose of API_EPE2 software is to extract, prepare, and encode data examples from CB513 data set taken from link: http://www.paraschorpa.com /project/evoca_prot/index.php. CB513 data set consists of 513 files with extension .all. These files contain information about protein sequences, but, for our algorithm, only information about primary and secondary structures is essential.

The first step in using our application software is to process all CB513 files and separate primary sequences and corresponding secondary structures. The result of this step is saved as file *IzdvCB513.txt* which consists of 513 non-homologous protein sequences and corresponding secondary structures. An example of this data is:

```
MVLSEGEWQLVLHVWAKVEADVAGHGQDILI...
CCCCHHHHHHHHHHHHHHGGGHHHHHHHHHH...
MNIFEMLRIDEGLRLKIYKDTEGYYTIGIGH...
CCHHHHHHHHHCCEEEEEECTTSCEEEETTE...
```

The second step is to encode protein sequences and corresponding secondary structures from 1-letter amino acid codes into numeric codes (Table 1) and result is saved as file *KodCB513.txt*. We also encode DSSP classes [9] into numeric codes (Table 2).

After that, we separate primary sequences from secondary structures and result is file for primary protein sequences *PrimCB513.txt*:

11 18 10 16 04 06 06 19 14 10 07 18 19 01 09 18 04 01 03 18 01…
11 12 08 05 04 11 10 15 08 03 04 06 10 15 10 09 08 20 09 03 17…

Table 1

Amin acids	Amino acids (referred in our language)	1-letter symbol	Code
Alanine	Alanin	A	01
Cysteine	Cistein	C	02
Aspartate	Asparaginska kiselina	D	03
Glutamate	Glutaminska kiselina	E	04
Phenylalanine	Fenilanin	F	05
Glycine	Glicin	G	06
Histidine	Histidin	H	07
Isolecine	Izoleucin	I	08
Lysine	Lizin	K	09
Leucine	Leucin	L	10
Methionine	Metionin	M	11
Asparagine	Asparagin	N	12
Proline	Proline	P	13
Glutamine	Glutamin	Q	14
Arginine	Arginin	R	15
Serine	Serin	S	16
Threonine	Treonin	T	17
Valine	Valin	V	18
Tryptophan	Triptofan	W	19
Threonine	Tirozin	Y	20

Table2

DSSP classes	Structures used in our algorithm	Code
H,G	Helix	01
E	Strand	02
B, I, S, T, C, L	Other structures	03

and file for secondary structures *SekCB513.txt* is:
3 03 03 03 01 01 01 01 01 01 01 01 01 01 01 01 01 01 01 01…
03 03 01 01 01 01 01 01 01 01 03 03 02 02 02 02 02 02 03 03 …

3. NEURAL NETWORK BASED ALGORITHM FOR SECONDARY STRUCTURE PREDICTION

3.1 Determination of neural network training sets batch matrices

Data files *PrimCB513.txt* and *SekCb513.txt* are used for creation of two matrices; the first one for input samples and second one for output samples. The samples in these two matrices are used for training of neural network. To make batch matrix of patterns from the amino acids sequences (stored in the file PrimCB513.txt) we use two functions implemented in MATLAB. The first function returns a vector which contains sequences of amino acids separated by number 0. For example, if file PrimCB513.txt contains three amino acids sequences in rows:

11 12 08 05 04 11 10 15

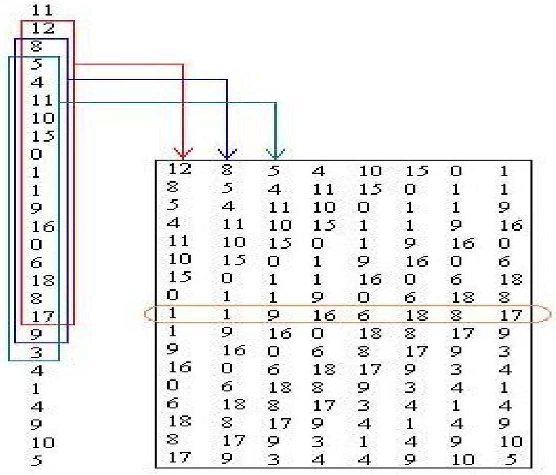
01 01 09 16

06 18 18 17 09 03 04 01 04 09 10 05

the first function (*prepare_data*) produces vector:

11 12 8 5 4 11 10 15 0 1 1 9 16 0 6 18 18 17 9 3 4 1 4 9 10 5

The second function (*prepare_pattern*) returns pattern batch matrix using one window. A window is a short segment of a complete protein string. In the middle of it there is an amino acid for which we want to predict secondary structure. Each column of matrix created by this function corresponds to one window in protein string (Figure 3).



12	8	5	4	10	15	0	1
8	5	4	11	15	0	1	1
5	4	11	10	0	1	1	9
4	11	10	15	1	1	9	16
11	10	15	0	1	9	16	0
10	15	0	1	9	16	0	6
15	0	1	1	16	0	6	18
0	1	1	9	0	6	18	8
1	1	9	16	6	18	8	17
1	9	16	0	18	8	17	9
9	16	0	6	8	17	9	3
16	0	6	18	17	9	3	4
0	6	18	8	9	3	4	1
6	18	8	17	3	4	1	4
18	8	17	9	4	1	4	9
8	17	9	3	1	4	9	10
17	9	3	4	4	9	10	5

Figure 2 Design of input pattern matrix taken from the string of encoded protein sequences

This window moves through protein, 1 amino acid at a time. Our prediction is made for central amino acid and if we encounter 0 (spacer between two proteins) at that position of window, than function *prepare_pattern* doesn't permit for that window to be placed into the pattern matrix. Then, with this function we transform 1-number code of amino acid into 20 number code. As you can see from the Figure 3, number 12 is transformed into 000000000001 00000000 and number 8 into 000000010000-00000000. Our matrix will require a neural network with 20 x17 input nodes (17 is length of the window) which have values 0 or 1.

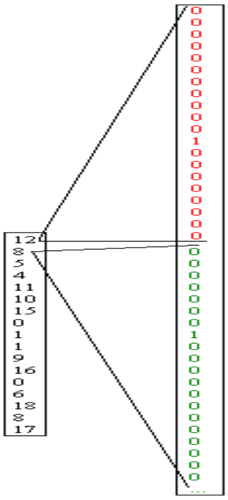


Figure 3 Transforming 1-number code into 20-number code

To make target matrix from the secondary structure (stored in the file SekCB513.txt), we use two functions implemented in MATLAB. The first function (*prepare_data*) returns a vector which contains only three types of numbers (1,2,3) and also 0-numbers corresponding to null-numbers at the input vector produced with function *prepare_data* (PrimCB513.txt). Second function *prepare_target* produces output matrix using the length of the window which is the same as the length of the input vector string. That function eliminates those secondary structures from the output vector which correspond to amino acids for which we don't make any prediction (the first eight and the last eight amino acids) in the input vector. As all elements in target vector must have values between 0 and 1 (activation function at output layer is *logsig*), function transforms 1-number code of secondary structure into 3-number code and generates target matrix whose column vectors denote secondary structure patterns: helix, strand, and other structures. For example, if secondary structure sequence is:

11231111
1132
112122211111
the function prepare_data produces the following string:
1123111101132011212221111.
The function prepare_target produces from the previous string the following target matrix:
1 1 0 0 1 1 0 1
0 0 0 1 0 0 1 0
0 0 1 0 0 0 0 0

3.2 Design, learning, and performance evaluation of neural networks for different size of windows

Prediction of secondary structure was implemented using non-linear neural network with three layers based on feed-forward supervised learning and back-propagation error algorithm [8][10]. Default values for window size, number of neurons in hidden layer and number of training epochs are presented in Table 3.

Table 3

Algorithm parameters	**Initial values**
Window sizes	13
Number of neurons in hidden layer	5
Number of training epochs	250

Instruction *creatennw* is used for designing neural networks with different sizes of windows (11, 13, 15, 17, and 19); instruction *createCB513w* is used for creation of training set, and training of neural network is started with instruction *trainw*. After that, we evaluated performances of neural networks using testing sets stored in files *PrimStrTestB.txt* and *SekStrTestB.txt.* Results are shown in Table 4.

Table 4

	training/testing sets	**window size (11-19)**	**Evaluated results**	
			Q3% (training set)	**Q3% (test set)**
1	trainsetw11/testsetw11	11	62,2150	60,9910
2	trainsetw13/testsetw13	13	62,4055	61,1011
3	trainsetw15/testsetw15	15	57,0756	54,7338
4	trainsetw17/testsetw17	17	62,5809	61,7053
5	trainsetw19/testsetw19	19	63,5312	62,5160
Average value Q3			61,5616	60,2094

3.3 Design, learning and performance evaluation of neural networks for different number of neurons in hidden layer

For this purpose we use the following instructions:
creatennhn (to design neural networks with different number of neurons in hidden layer),
trainhn (to train neural networks), and
accuracyhn (for performance evaluation). Results are shown in Table 5.

Table 5

	Number of neurons in hidden layer (1-13)	**Test**	
		Q3% (training set)	**Q3% (testset)**
1	2	44.1607	41.9736
2	3	63.2946	61.5593
3	4	63.2101	62.2373
4	5	63.5312	**62.5160**
5	6	62.8499	61.4689
6	7	63.6632	62.1544
7	8	63.6323	62.3352
8	9	63.6168	62.1695
9	10	63.4052	61.8531
10	11	63.5086	62.1544
11	12	63.4111	62.0866
12	13	63.7809	62.3051
Average value Q3		61,8387	60,4011

3.4 Design, learning and performance evaluation of neural networks for different number of epochs

At the end we used five neurons in hidden layer, window size 19 and different number of epochs using instructions *traine*. Results are shown in Table 6.

3.5 Performance evaluation of neural networks using nonhomologus data test

CB513, data set in which there is no sequence similarity between protein sequences, was divided into two sets:

- 413 training protein sequences (stored in *PrimTrain413.txt* data file and *SekTrain413 data file*), and
- 100 test protein sequences (stored in *PrimTest100.txt* and *SekTest100.txt* data file).

In MATLAB a software package *nonhomtest* was created with the following functions:

- design of input data (samples) matrix using *PrimTrain413.txt* data file, and design of output data matrix using *SekTrain413* data file,
- design of neural network based on 5 neurons in hidden layer, and using window size 19,
- training of designed neural network in time interval of 4000 epochs,
- design of input data (samples) matrix using *PrimTest100.txt* data file, and design of output data matrix using *SekTest100* data file, and
- accuracy evaluation of our neural networks using .

Accuracy of a neural network prediction evaluated with non-homologous test set is **62.7253**.

Table 6

	Number of neurons in hidden layer (1-13)	**Test**	
		Q3% (training set)	**Q3% (test set)**
1	100	44.5602	43.0508
2	150	57.8345	56.1582
3	200	63.0461	61.9962
4	250	63.5312	62.5160
5	300	63.5229	62.3879
6	500	63.5550	62.4633
7	1000	63.7357	62.3879
8	2000	64.3885	62.5838
9	3000	64.7369	62.8173
10	4000	64.8546	**62.8776**
11	5000	64.8914	62.8399
12	10000	64.5740	61.9736
Average value Q3		61.9539	60.3377

4. CONCLUSION

In this paper we have demonstrated the power of the artificial neural networks in extracting information from the protein structure database and in predicting secondary structural features from sequence alone. For design of neural network for prediction of secondary protein structure we used the modified algorithm API_EPE [8]. In that work [8] neural network was evaluated with protein set without considering existence of similarity between protein sequences. In this study, we used training set (CB513) in which there are no sequence similarities between protein sequences. But, in test set we did not consider homology between protein sequences and that test set was taken from PDBFIND2 data base. Because of that we cannot assure that results of predictions 62.8776 and 63.6261 [8] are fully objective. The solution in this study is based on dividing CB513 non-homologous data set into two subsets. The first one consists of 413 non-homologous protein sequences and was used for training of our neural network. The second subset consists of 100 non-homologous protein sequences and was used for test of our neural networks. Accuracy of prediction for these two subsets is 62.7253, and we can assert that this is objective. The achieved exactness is relatively smaller compared to the study which used neural network training and test sets without verification of protein homology.

REFERENCES

[1] V. I. Lim, Algorithms for prediction of α helices and β structural regions in globular proteins, J. Mol. Biology, 1974;88:873-894.

[2] J. Garnier, D. J. Osguthorpe, and B. Robson. Analysis and implications of simple methods for predicting the secondary structure of globular proteins, J. Mol. Biology, 1978;120: 97-120.

[3] N. Qian and T. Sejnowski, Predicting the secondary structure of globular proteins using neural network models. J. Mol. Biology,1988; 202:865-884, Available from: http://brahms.cpmc.columbia.edu/publications/protein.pdf

[4] B. Rost, C. Sander, Prediction of protein secondary structure at better than 70% accuracy, J. Mol. Biology, 1993;232:584-599.

[5] A. M. Campbell, L. J.Heyer, Discovering Genomics, Proteomics and Bioinformatics,CHSL Press, Benjamin Cummings, San Francisco, 2003,

[6] B. R. Rost, C. Sander, and R. Schneider, Redefining the goals of protein secondary structure prediction, J. Mol. Biology, 1994; 235:13-26.

[7] Cuff J. A., Barton G.J., Evaluation and Improvement of Multiple Sequence Methods for Protein Secondary Structure Prediction, PROTEINS: Structure, Function, and Genetics, 1999;34:508–519, Available from: http://binf.gmu.edu/vaisman/csi731/pr99-cuff.pdf

[8] Z.Avdagic, E.Purisevic, Feed-Forward Neural Network for Protein Structure Prediction, Proccedings:Cybernetics and Systems, Volume 1, Vienna; Austrian Society for Cybernetic Study; 2006, 198p.

[9] David W.Mount, Bioinformatics: Sequence and Genome Analysis, New York; CHSL Press;2001, 13 p.

[10] S. R.Holbrook, S. M. Muskal, S.H. Kim, Predicting Protein Structural Features with Artificial Neural Networks, ed. L. Hunter Artificial Intelligence and Molecular Biology, Massachusetts; The MIT Press , Massachusetts Institute of Technology; 1993, 161p.