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Machine Learning and Graph Theory Approaches for Classification and Prediction of Protein Structure

Gulsah Altun

MACHINE LEARNING AND GRAPH THEORY APPROACHES FOR CLASSIFICATION AND PREDICTION OF PROTEIN STRUCTURE

by

GULSAH ALTUN

Under the direction of Dr. Robert W. Harrison

ABSTRACT

Recently, many methods have been proposed for the classification and prediction problems in bioinformatics. One of these problems is the protein structure prediction. Machine learning approaches and new algorithms have been proposed to solve this problem. Among the machine learning approaches, Support Vector Machines (SVM) have attracted a lot of attention due to their high prediction accuracy. Since protein data consists of sequence and structural information, another most widely used approach for modeling this structured data is to use graphs. In computer science, graph theory has been widely studied; however it has only been recently applied to bioinformatics. In this work, we introduced new algorithms based on statistical methods, graph theory concepts and machine learning for the protein structure prediction problem. A new statistical method based on z-scores has been introduced for seed selection in proteins. A new method based on finding common cliques in protein data for feature selection is also introduced, which reduces noise in the data. We also introduced new binary classifiers for the prediction of structural transitions in proteins. These

new binary classifiers achieve much higher accuracy results than the current traditional binary classifiers.

INDEX WORDS: algorithm, machine learning, graph theory, support vector machines, feature selection, protein structure prediction

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by

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DEDICATION

To my father Zikri Altun, my mother Aynur Altun
and my sister Gokcen Altun Ciftcioglu

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LIST OF ACRONYMS

1. SVM - Support Vector Machines
2. PSSM - Position-Specific Scoring Matrix
3. NMR - Nuclear Magnetic Resonance
4. BLAST - The Basic Local Alignment Search Tool
5. CASP - Critical Assessment of Techniques for Protein Structure Prediction
6. PSI-BLAST - Position Specific Iterative BLAST
8. PISCES - Protein Sequence Culling Server
9. HSSP - Homology-derived Secondary Structure of Proteins
10. PDB - Protein Data Bank
11. DSSP - Dictionary of Secondary Structure of Proteins
12. BLOSUM - Blocks of Amino Acid Substitution Matrix substitution matrix

CHAPTER 1

Introduction

Recently, many methods have been proposed for the classification and prediction problems in bioinformatics [9][38][43]. One these problems is the protein structure prediction problem. Solving the protein structure prediction problem is one of the ten most wanted solutions in protein bioinformatics [65]. Proteins are the major components of living organisms and are considered to be the working and structural molecules of cells and they are composed of building-block units called amino acids [34][45]. These amino acids dictate the structure of a protein [72].

Many machine learning approaches and new algorithms have been proposed to solve the protein structure prediction problem [5][8][16][14][41][60]. Among the machine learning approaches, Support Vector Machines (SVM) have attracted a lot of attention due to its high prediction accuracy. Since protein data consists of sequence and structural information, another widely used approach for modeling this structured data is to analyze it as graphs. In computer science, graph theory has been widely studied; however it has been recently applied to bioinformatics. In this work, we introduced new algorithms based on statistical methods, graph theory concepts and machine learning for the protein structure prediction problem.

In this work, we introduced new algorithms based on statistical methods, graph theory concepts and machine learning for the protein structure prediction problem. We introduced a

new statistical method based on z-scores has been introduced for seed selection in protein data. We also developed a new method based on finding common cliques in protein data for feature selection. This method reduces noise in the data. We also introduced new binary classifiers for the prediction of structural transitions in proteins. Our new binary classifiers achieve much higher accuracy results than the current traditional binary classifiers.

In the following, a short description of the methods and results that are described in each chapter of this dissertation is given:

In chapter 2, the problem definitions and related work is presented. This chapter gives a general background for the methods that we propose in this work. In this chapter, proteins are introduced in detail. Then, the formal problem formulation for protein structure prediction is given. We also give background of two machine learning approaches; support vector machines and random forests. The mathematical theories behind these two approaches are explained in detail. A brief introduction to feature selection is given and some related work is explained. Then, a brief background to graph theory is given.

In chapter 3, we propose a new algorithm based on a statistical approach using z-scores that maximizes the likelihood of seeds sharing the same local structure in both the query and known protein sequences. A seed is a short contiguous or patterned match of amino acids of two or more protein sequences that can be extended to find alignments between these proteins. We evaluated our algorithms on the 2290 protein sequences in the PISCES (Protein sequence culling server) database [69]. Our new algorithm results in an effective a priori estimate of seed structural quality which results in finding better query seeds in a BLAST (The Basic Local Alignment Search Tool) search [3].

In this study, the factors involved in the accurate selection of seeds for protein sequence alignments were explored. It is possible to identify seeds that are likely to share structural similarity with a meaningful *a priori* assessment of accuracy by using a profile-clustered profile approach. We used high order information identified by clustering and showed that it is reliable in small scales. We found that look-up of this clustered sequence-based seeds for the best match works much better than look-up of individual frequency profile of each seed in the database. The predictive ability of these clusters suggests that there are distinct sequence-structure seeds. The dramatic improvement found by using high quality clustered profiles shows that higher order descriptions of sequence similarity are required for accurate results in the prediction of protein structure. This suggests that PHI-BLAST like algorithms can be substantially improved if the database is clustered first. Our results show that it is possible to select seeds when sequence windows are clustered and average profiles of these clusters are used for calculating similarity measure.

In chapter 4, we propose two hybrid kernels SVM_{SM+RBF} and $SVM_{EDIT+RBF}$. The goal of this work is to find the best kernel function that can be applied to different types of problems and application domains. We propose two hybrid kernels SVM_{SM+RBF} and $SVM_{EDIT+RBF}$ [5]. SVM_{SM+RBF} is designed by combining the best performed radial basis function (RBF) kernel with substitution matrix (SM) based kernel developed by Vanschoenwinkel and Manderick [66]. $SVM_{EDIT+RBF}$ is designed by combining the edit kernel devised by Li and Jiang [47] with the RBF kernel. In our approach, two hybrid kernels are devised by combining the best performed RBF kernel with substitution matrix (SM) based kernel [66] and with edit kernel [47]. We tested these two kernels on the CB513 and RS126 protein datasets for the protein secondary structure problem. Two data sets were used in evaluating our system. The RS126

dataset consists of 126 protein chains, was presented by Rost and Sander [61]. The CB513 dataset by Cuff and Barton contains 513 proteins [22]. Our results were 91% accuracy on H/E binary classifier. In this case, the information in the substitution matrix reinforces the information in the RBF on PSSM profiles. However, this is not true with the edit distance. These results show us that the data are consistent when substitution matrix is used and not consistent when edit distance is used. The edit distance kernel gives good results in [47], but not when used with our dataset in this work. Our results show that it is critically important to use mutually consistent data when merging different distance measures in support vector machines.

In chapter 5, we propose a new algorithm that uses a graph theoretical approach which finds cliques in the non-position specific evolutionary profiles of proteins obtained from BLOSUM62. Even though, graph theory concepts have been around for more than a century, its concepts are just newly being explored for applying to biology [13][67]. The clique search algorithm was applied to find all the cliques with the different threshold values. In this work, we propose an algorithm that used a graph theory approach for feature selection. First, we apply this algorithm on BLOSUM62 matrix and then based on the feature set produced by the algorithm; we use this feature set for condensing the PSSM matrix. Next, based on the newly designed algorithm, final cliques were determined. By merging the vertices within the same clique into one, the original feature space is reduced. Finally, this reduced feature set was applied to random forests and the performance was compared with the unreduced counterpart. These cliques the features selected by this algorithm are used for condensing the position specific evolutionary information obtained from PSI-BLAST. Our results show that

we are able to save significant amount of space and time and still achieve high accuracy results even when the features of the data are 25% reduced.

In chapter 6, we introduce a novel encoding scheme and a computational method using machine learning for prediction starts and ends of secondary structure elements. Most computational methods have been developed with the goal to predict the secondary structure of every residue of a given protein sequence. However, instead of targeting to predict the structure of each and every residue, a method that can correctly predict where each secondary structure segment (such as alpha-helices, beta-sheets or coils) in a protein starts and ends could be much more reliable since less number of predictions are required. Our system makes only one prediction to determine whether a given sequence segment is the start or end of any secondary structure H, E or C, whereas the traditional methods must be able to predict each and every residue's structure correctly in the segment to be able to make that decision. We compared the traditional existing binary classifiers, to the new binary classifiers proposed in this work and achieved a much higher accuracy than the traditional approach.

In chapter 7, we give future work. As a future work, our clique finding algorithm can be enhanced for the newly proposed encoding scheme in chapter 6. Finding common amino acid patterns in transition boundaries could be useful in making our feature selection algorithm more robust and accurate. These common patterns will be searched when a prediction is being made. Where in the protein these common patterns occur is also important. Depending on whether at the beginning of a sequence or end of a sequence is, the transition boundary could be changed drastically. A new encoding scheme will be developed to represent this information as well. This is one of the future problems that can be explores in the future.

In chapter 8, we give a conclusion where we summarize our work. The expected contribution of this dissertation work involves two aspects: first, we developed new algorithms drawing from graph theory and machine learning for structured data prediction. In protein structure prediction, we encountered too many negative data and just a few positive examples. The datasets are huge and these problems are shared by the data in many applications. We tested our methods on protein structure data; our methods, however, are more general and were tested for different data and applications such as micro array and gene data. We propose methods for predicting protein secondary structure and detecting transition boundaries of secondary structures of helices (H), coils (C) and sheets (E). Detecting transition boundaries instead of the structure of individual residues in the whole sequence is much easier. Thus, our problem is reduced to the problem of finding these transition boundaries.

CHAPTER 2

Problem Definitions and Related Work

In this chapter, problem definitions, motivation and related work are presented. This chapter gives a general background for the methods that we propose in chapters 3, 4, 5 and 6.

2.1. Prediction of protein structure

Proteins are polymers of amino acids containing a constant main chain (linear polymer of amino acids) or backbone of repeating units with a variable side chain (sets of atoms attached to each alpha-carbon of the main chain) attached to each [44]. Proteins play a variety of roles that define particular functions of a cell [44]. They are a critical component of all cells and are involved in almost every function performed by them. Proteins are building blocks of the body controls; they help communicating with cells and transport substances. Biochemical reactions which are done by enzymes also contain protein. The transcription factors that turn genes on and off are proteins as well.

A protein is primarily made up of amino acids, which determine its structure. There are 20 amino acids that can produce countless combinations of proteins [34][55]. There are four levels of structure in a protein: the first level is the primary structure of the protein, which is its amino acid sequence. A typical protein contains 200-300 amino acids. The second level is the secondary structure, which is formed of recurring shapes called helices, strands, and coils

as shown in Figure 2.1. Many proteins contain helices and strands. The third level is the tertiary structure of a protein which is the spatial assembly of helices and sheets and the pattern of interactions between them. This is also called the folding pattern of a protein. Many proteins contain more than one polypeptide chain; the combinations two or more polypeptide chains in a protein make up its quaternary structure [10][20]. The protein in Figure 2.1 is a CASPase 7 protein borrowed from the Weber lab in the Georgia State University (GSU) Biology department.

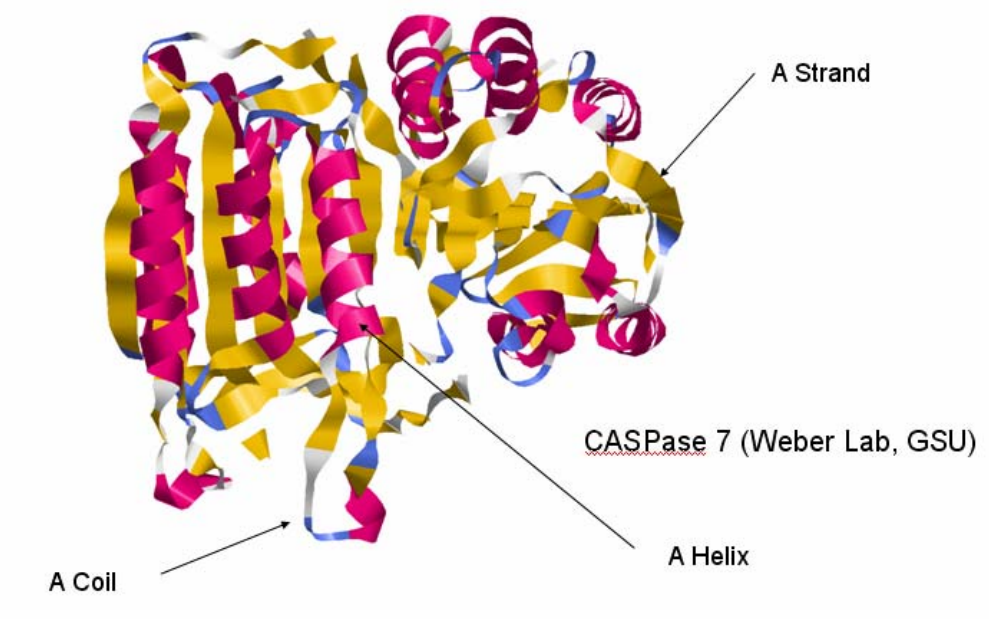


Figure 2.1 CASPASE 7 protein

Proteins interact with DNA (Deoxyribonucleic acid), RNA (Ribonucleic acid) and other proteins in their tertiary and quaternary state. Therefore, knowing the structure of a protein is crucial for understanding its function.

Recently, large volumes of genes have been sequenced. Therefore, the gap between known protein sequences and protein structures that have been experimentally determined is growing exponentially. Today, in Protein Data Bank (PDB) [11] there are over 1 million proteins whose amino acid sequence are known; however, only a very little fraction (~50,000) of these protein structures are known [8][11]. The reason for this gap is that Nuclear Magnetic Resonance (NMR) and x-ray crystallography techniques take years to determine the structure of one protein. Therefore, having computational tools to predict the structure of a protein is very important and necessary. Even though most of the computational methods proposed for protein structure prediction do not give 100% accurate results, even an approximate model can help experimental biologists guide their experiments. Predicting the secondary and tertiary structure of a protein from its amino acid sequence is one of the important problems in bioinformatics. However, with the methods available today, protein tertiary structure prediction is a very hard task even when starting from the exact knowledge of protein backbone torsion angles [12]. It is also suggested that protein secondary structure delimits the overall topology of the proteins [50]. It is believed that predicting the protein secondary structure provides insight into and an important starting point for the prediction of the tertiary structure of the protein, which leads to understanding the function of the protein. Recently, there have been many approaches to reveal the protein secondary structure from the primary sequence information [19][27][56][57][58][59] [75][76].

2.2. Protein secondary structure prediction problem formulation

In this work, we adopted the most generally used DSSP secondary structure assignment scheme [39]. The DSSP classifies the secondary structure into eight different classes: H (α-helix), G (3_{10}-helix), I (π-helix), E (β-strand), B (isolated β-bridge), T (turn), S (bend), and - (rest). These eight classes were reduced for the purposes of this dissertation into three regular classes based on the following method: H, G and I to H; E to E; all others to C. In this work, H represents helices; E represents sheets and C represents coils.

The problem formulation is stated as:

Given: *A protein sequence $a_1a_2...a_N$, secondary structure prediction*

Find: *The state of each amino acid a_i as being either H (helix), E (beta strand), or C (coil).*

The quality of secondary structure prediction is measured with a "3-state accuracy" score called Q_3. The Q_3 formula is the percent of residues that match reality as shown below in equation 2.1.

$$Q_3 = \frac{\sum_{i \in \{H,E,C\}} \#\ of\ \ residues\ \ correctly\ \ predicted_{\ i}}{\sum_{i \in \{H,E,C\}} \#\ of\ \ residues\ \ in\ class\ \ i} \quad (2.1)$$

Q3 is one of the most commonly used performance measures in protein secondary structure prediction. Q3 refers to the three-state overall percentage of correctly predicted residues.

2.3. Previous work on protein secondary structure prediction

The protein secondary structure prediction problem has been studied widely for almost a quarter of a century. Many methods have been developed for the prediction of the secondary structure of proteins. In the initial approaches, secondary structure predictions were performed on single sequences rather than families of homologous sequences [26]. The methods were shown to be around 65% accurate. Later, with the availability of large families of homologous sequences, it was found that when these methods were applied to a family of proteins rather than a single sequence, the accuracy increased well above 70%. Today, many proposed methods utilize evolutionary information such as multiple alignments and PSI-BLAST profiles [2]. Many of these methods that are based on Neural networks, SVM and hidden Markov models have been very successful [5][8][16][14][41][60]. The accuracy of these methods reaches around 80%. An excellent review on the methods for protein secondary structure prediction has been published by Ross [60].

Recently, there has been an increase in pattern-based approaches for protein secondary structure prediction due to their high accuracy values, which are mostly above 80%. Among these, machine learning methods SVM, decision trees and random forests have been attracting a lot of attention. In this work, we propose a new algorithm that adapts a graph theory approach combined with random forests for the secondary structure prediction problem and feature selection. In section 2.4 we give a brief introduction to random forests.

2.4. Random forests

Random forests were proposed by Leo Breiman [14]. Random forests are a combination of decision trees; each tree is grown from a randomly sampled set of the training data as shown in Figure 2.2.

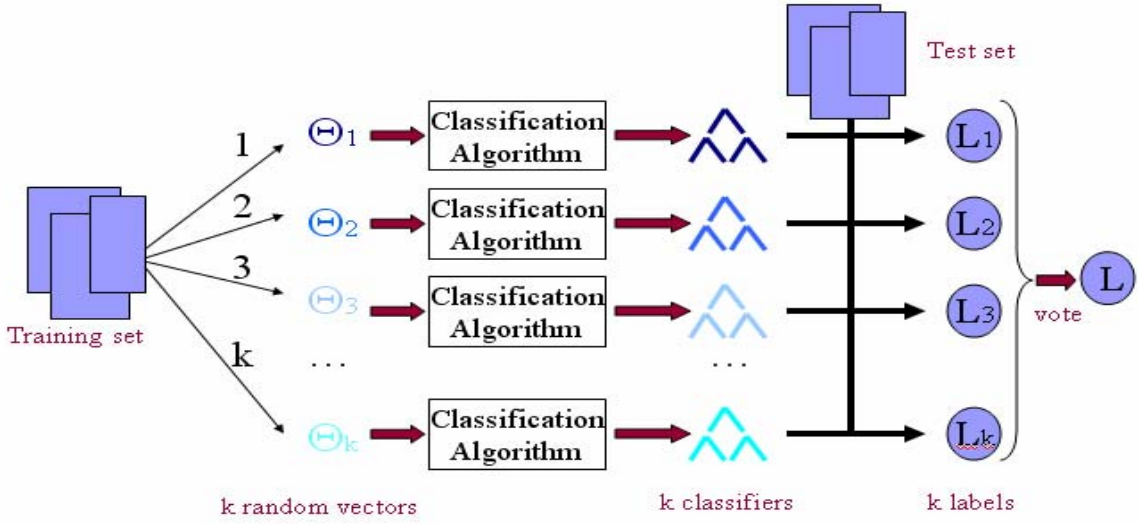


Figure 2.2 Random forests

Each of the classification trees (k classifiers) is built using a bootstrap sample of the data. Each tree outputs a class for a given set of test data, and the test data is labeled with the class that has the majority of the votes from these trees. Given M features in a training set, the best splitting feature is determined for each decision tree in the random forest from a randomly selected subspace of m features at each decision node. The optimal value of m is usually the square root of M; however, this m value also depends on the strength and correlation of the trees. The user has to specify the m value accordingly.

Random forests use both bagging and random variable selection for tree building. There is no pruning. Bagging and random variable selection result in low correlation of the

individual trees, which yields better classification [14][25]. Random forests do not overfit and show comparable results to other machine learning approaches such as SVM. It is a robust method concerning the noise and the number of attributes. Generated forests in random forests can be saved for future use on other data.

2.5. Random forest software

The random forests software used in this work is an implementation of random forests [15] written in extended Fortran 77.

2.6. Support Vector Machines

The Support Vector Machines (SVM) algorithm is a modern learning system designed by Vapnik and Cortes [68]. Based on statistical learning theory which explains the learning process from a statistical point of view, the SVM algorithm creates a hyperplane that separates the data into two classes with the maximum margin. Originally, it was a linear classifier based on the optimal hyperplane algorithm. However, by applying the kernel method to the maximum-margin hyperplane, Vapnik and his colleagues proposed a method to build a non-linear classifier. In 1995, Cortes and Vapnik suggested a soft margin classifier, which is a modified maximum margin classifier that allows for misclassified data. If there is no hyperplane that can separate the data into two classes, the soft margin classifier selects a hyperplane that separates the data as cleanly as possible with maximum margin [17].

SVM learning is related to recognizing patterns from the training data [1][23]. Namely, we

estimate a function f: $R_N \rightarrow \{\pm 1\}$, based on the training data which have an N-dimensional pattern $\boldsymbol{x_i}$ and class labels y_i. By imposing the restriction called Structural Risk Minimization (SRM) on this function, it will correctly classify the new data (x, y) which has the same probability distribution P(x,y) as the training data. SRM determines the learning machine that yields a good trade-off between low empirical risk (mean error over the training data) and small capacity (a set of functions that can be implemented by the learning machine).

In the linear soft margin SVM which allows some misclassified points, the optimal hyperplane can be found by solving the following constrained quadratic optimization problem.

$$\min_{w,b,\varepsilon} \ \frac{1}{2}\|w\|^2 + C\sum_{i=1}^{l}\varepsilon_i \qquad (2.2)$$

$$s.t. \ \ y_i\,(w \bullet x_i + b) \geq 1 - \varepsilon_i \ \ \varepsilon_i > 0 \qquad i = 1,....,l$$

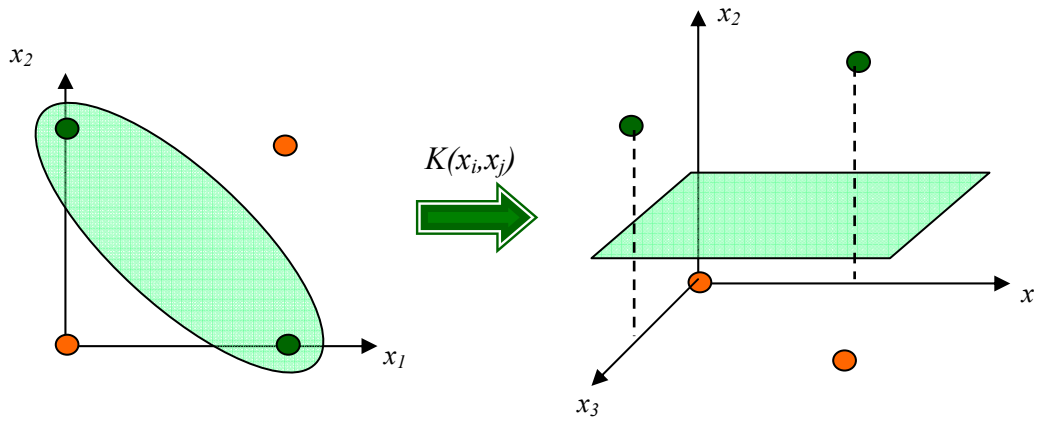
Where, $\boldsymbol{x_i}$ is an input vector, y_i = +1 or -1 based on whether x_i is in a positive class or negative class, 'l' is the number of training data, '*w*' is a weight vector perpendicular to the hyperplane and '*b*' is a bias which moves the hyperplane parallel to itself. Also '*C*' is a cost factor (penalty for misclassified data) and ε is a slack variable for misclassified points. The resulting hyperplane decision function is

$$f(x) = sign(\sum_{i=1}^{SV}\alpha_i y_i\,(x \bullet x_i) + b) \quad (2.3)$$

where, α_i is a Lagrange multiplier for each training data. The points $\alpha_i > 0$ lie on the boundary of the hyperplane and are called 'support vectors'. In Eq. (2.2) and (2.3), it is observed that both the optimization problem and the decision function rely on the dot products between each pattern.

In the non-linear SVM, the algorithm first maps the data into high-dimensional feature

space (F) via the kernel function φ(•):X→F and constructs the optimal separating hyperplane there using the linear algorithm as can be seen in Figure 2.3.



(a) Not separable by linear boundary (b) Linearly separable

Figure 2.3 Non-linear SVM mapping

According to Mercer's theorem, any symmetric positive definite matrix can be regarded as a kernel function. The positive definite kernel is defined as follows [23]:

Definition 1. Let X be a nonempty set. A function k(•, •):

X x X → R is called a positive definite kernel if k(•, •) is symmetric and for all n ∈N, $x_1,\ldots, x_n \in X$ and $a_1, \ldots, a_n \in R$.

The traditional positive definite kernel functions are the following:

$$K(x, y) = (x \bullet y + 1)^p \qquad (2.4)$$

$$K(x, y) = e^{-\gamma \| x - y \|^2} \qquad (2.5)$$

$$K(x, y) = \tanh(kx \bullet y - \delta) \qquad (2.6)$$

Eq. (2.4) is a polynomial, Eq. (2.5) is a Gaussian radial basis function (RBF), and Eq. (2.6) is a two-layer sigmoidal neural network kernel. Based on one of the above kernel functions,

the final non-linear decision function has the form

$$f(x) = sign\left(\sum_{i=1}^{SV} \alpha_i y_i K(x \bullet x_i) + b\right) \qquad (2.7)$$

The choice of proper kernel is critical to the success of the SVM. In the previous protein secondary structure prediction studies, a radial basis function worked best [32][33].

2.7. SVM software

SVM^{light} is an implementation of Support Vector Machines (SVM) in C [36]. In this work, we adopt the SVM^{light} software, which is an implementation of Vapnik's Support Vector Machines [67]. This software also provides methods for assessing the generalization performance efficiently.

SVM^{light} consists of a learning module (`svm_learn`) and a classification module (`svm_classify`). The classification module can be used to apply the learned model to new examples.

The format of training data and test data input file is as follows:

<line> .=. <target> <feature>:<value> <feature>:<value> ...

<target> .=. +1 | -1 | 0 | <float>

<feature> .=. <integer> | "qid"

<value> .=. <float>

For classification, the target value denotes the class of the example. +1 and -1 as the target values denote positive and negative examples, respectively.

2.8. Feature selection

Analysis with a large number of variables requires a large amount of memory and computation time. The problem of selecting a subset of relevant features in a large quantity of data is very important. Feature selection is a process commonly used in machine learning, where a subset of the features available from the data is selected for the learning algorithm. Feature selection is often necessary where it is computationally infeasible to use all available features. One of the main benefits of feature selection is that it reduces training and storage requirements. Also, a good feature selection mechanism can improve the classification by eliminating noisy or non-representative features.

There has been a lot of research on feature selection. Birzele and Kramer [12] have used a new representation for protein secondary structure prediction based on frequent patterns, which gives competitive results with the current techniques. Shi and P. N. Suganthan [63] investigated feature analysis for the prediction of the secondary structure of protein sequences using support vector machines (SVM) and the K-nearest neighbors algorithm (KNN). They applied feature selection and scaling techniques to obtain a number of distinct feature subsets. Their experimental results show that the feature subset selection improves the performance for both SVM and KNN.

Kurgan and Homaeian [44] describe a new method for predicting protein secondary structure content based on feature selection and multiple linear regression. The application of

feature selection and the novel representation result in a 14-15% error rate reduction when compared to results where normal representation is used. Their prediction tests also show that a small set of 5-25 features is sufficient to achieve accurate predictions for the helix and strand content of non-homologous proteins. Karypis proposes a new encoding scheme and better kernels for the protein secondary structure problem [40]. In the proposed new coding scheme, both position-specific and non-position-specific information are combined for the representation of each protein sequence. In this work, we compare this new encoding scheme with many different encoding schemes and present the results.

Su *et al.* [64] have used a condensed position-specific scoring matrices with respect to physicochemical properties (PSSMP), where the matrices are derived by merging several amino acid columns of a PSSM matrix sharing a certain property into a single column. Their experimental results show that the selected feature set improves the performance of a classifier built with Radial Basis Function Networks (RBFN) when compared with the feature set constructed with PSSMs or PSSMPs that simply adopt the conventional physicochemical properties. In order to get an effective and compact feature set for this problem, they propose a hybrid feature selection method that inherits the efficiency of univariant analysis and the effectiveness of the stepwise feature selection that explores combinations of multiple features. They decompose each conventional physicochemical property of amino acids into two disjoint groups which have a propensity for order and disorder, respectively. Then, they show that some of the new properties perform better than their parent properties in predicting protein disorder.

2.9. Graph Theory

In mathematics and computer science, graph theory is the study of *graphs* –mathematical structures used to model pair-wise relations between objects from a certain collection. Graph algorithms are good for data mining and modeling; additionally, it is powerful to have a graphic statistic model [29][70].

Many problems today can be stated in terms of a graph. Since the properties of graphs are well-studied in computer science, many algorithms exists to solve problems that are posed as graphs. Recently many bioinformatics problems have been studied using graph theory .Usually biological data is represented as mathematical objects (strings, sets, graphs, permutations, etc.), then biological relations are mapped into mathematical relations, and then the biological question is formulated. An excellent survey on graph theory and protein structures can be found in [61]. Although the topic is more than two centuries old, only recently has it gained momentum and been routinely used in various branches of science and engineering.

CHAPTER 3

A New Seed Selection Algorithm that Maximizes Local Structural Similarity in Proteins

All homology methods and many *ab initio* methods assume that similar sequences have similar structures [18][53][59]. Recent work suggests that finding short contiguous or patterned matches, called seeds or words, can be extended to find alignments [52]. Similarity searches based on the strategy of finding short seed matches have been widely studied, and many programs have been developed using this approach. One of the most popular programs is BLAST (Basic Local Alignment Search Tool), which has been cited over 10000 times over the last decade; the BLAST server currently receives about 100000 hits per day [3][56].

Given a query protein or DNA sequence along with a pattern (query sequence) occurring within the sequence, the Pattern Hit Initiated BLAST (PHI-BLAST) program searches a protein database for other instances of the query sequence in order to build local alignment [2][74]. This is because of the assumption that a good alignment is likely to contain high-scoring pairs of seeds. Many methods have been proposed to find more optimal seeds by using gapped alignments or position-specific scoring matrices [2][18][21][24][28][30] [46][48][60][69][73]. However, some of these methods select seeds by scanning each sequence window of a given size k in the database one by one, which can result in many false positives due to the large number of sequence windows in a protein database.

Therefore, it is crucial to evaluate the factors in selecting seeds to minimize the number

of false positives. In this work, we explore the reliability of z-score statistics when used on sequence vs. profile, profile vs. profile and profile vs. clustered profile approaches to define seeds.

Sequence vs. profile methods use a single profile for the first sequence and the second sequence to select scores from the profile. For example, PSI-BLAST derives profile sequence alignments and then uses the query sequence to find the score [2]. In profile vs. profile methods, the two profiles are compared. For example, the Fold and Function Assignment System (FFAS) server uses the dot product of the two profiles when aligning protein sequences [35]. Neither sequence vs. profile nor profile vs. profile methods has any means of assessing the statistical significance of the profile. Clustering the profiles as a preprocessing step extracts profiles that are conserved in sequence space and that are, thus, likely to correspond to conserved structure or function in the proteins. The Profile vs. Clustered profile algorithm, suggested in this work, can take advantage of this statistical significance. The sequence clusters can be assigned a quality based on their internal statistical consistency; this quality strongly correlates with the structural similarity in the proteins that contain them.

3.1 Experimental setup

The dataset used in this work includes 2290 protein sequences obtained from the Protein Sequence Culling Server (PISCES) [62][69]. Protein sequences in this database do not share more than 25% sequence similarity in this database. We also used the sliding window scheme. When predicting or analyzing some characteristics of an amino acid, a window that is centered with that particular amino acid is used. In the sliding window scheme, every amino acid in the protein becomes a center and a window becomes one training pattern for

predicting the structure of that residue. All the sliding windows with nine successive and continuous residues are generated from protein sequences. The width of nine residues was chosen to be representative of the size of protein-folding motifs. While the optimal sizes are not constant and may be either larger or smaller than nine residues, this is a useful approximation and removes sample size bias from the analysis. The frequency profile from a database of homology-derived secondary structures of proteins (HSSP) is constructed based on the alignment of each protein sequence from the Protein Data Bank (PDB) in which all the sequences are considered homologous in the sequence database [54][51]. Using the sliding window technique, 500,000 sequence windows are generated. Each sequence window is represented by either the amino acid residue or the 9x20 HSSP profile matrix, depending on the method applied. Twenty columns represent the 20 amino acids and 9 rows represent each position of the sliding window.

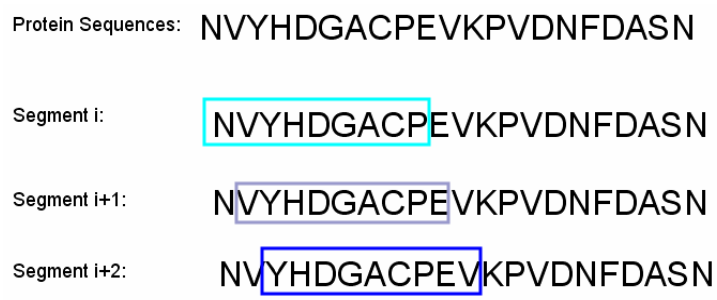


Figure 3.1 Sliding window representation

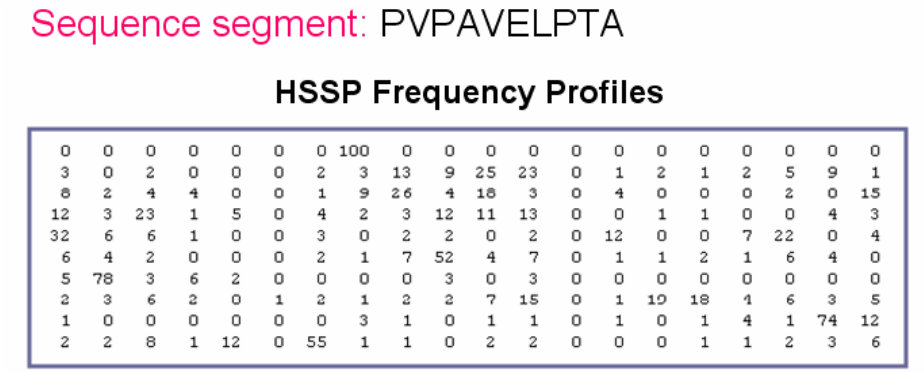


Figure 3.2 HSSP representation of sequence profiles

3.2 Sequence vs. Profile Algorithm

In the Sequence vs. Profile algorithm, each sequence window in the database is represented by its frequency profile produced by the multiple sequence alignment. However, the query sequence is represented solely by its amino acid residues. The scores were calculated for a window width of 9 residues. Z-scores were used to place the results in a constant scale with respect to the standard deviation. Thus two samples with similar z-scores have similar statistical significance. The formula to calculate the score for a sequence window of size 9 is given in the following equation:

$$z-score = \sum_{i=1}^{9} \frac{Freq_i - Avg_i}{Std_i} = \sum_{i=1}^{9} individual\ \ z-score \quad (3.1)$$

$Freq_i$: The frequency of the i^{th} amino acid of the sequence window in the sequence profile database

Avg_i : The average value of the the i^{th} amino acid in the entire database.

Std_i : The standard deviation value of the i^{th} amino acid in the entire database.

After each sequence window in the database is assigned a z-score, the sequence window which receives the highest z-score after the comparison process is considered to be the best match for the given query.

3.3 Profile vs. Profile Algorithm

In the Profile vs. Profile algorithm, a given query amino acid sequence window is represented by the frequency profile rather than its amino acid sequence representation, as was done in the Sequence vs. Profile method. The sequence window in the database having a frequency profile closest to the frequency profile of a given amino acid sequence window is considered to be the best match for the Profile vs. Profile method.

$$Avg = \frac{\sum_{i=1}^{N} score_i}{N} \quad (3.2)$$

$$Std = \frac{\sum_{i=1}^{N} (score_i - Avg)^2}{N} \quad (3.3)$$

$score_i$: The score assigned to the i^{th} sequence segment in the sequence profile database.

$$z - score = \frac{score_i - Avg}{Std} \quad (3.4)$$

3.4 Profile vs. Clustered Profile Algorithm

In the Profile vs. Clustered Profile algorithm, we propose a cluster-based approach which is different from the previous two methods. In this algorithm, initially all the sequence windows in the database are classified into different sequence-based clusters by the K-means clustering algorithm [75]. We used the K-means algorithm because it produces many high quality clusters and because it is an efficient way to cluster a huge dataset such as PISCES [75]. After all sequence windows are clustered based on their sequence similarity using HSSP profiles, each cluster was assigned an average profile that represents that cluster.

After finding the clusters, each cluster was ranked based on the secondary structure similarity of each sequence window that they contain. Based on this ranking the clusters were divided into high quality clusters, average quality clusters and low quality clusters. A cluster was ranked as high quality if at least 70% of the sequence windows that the cluster contains shared more than 70% secondary structure similarity. Similarly, if at most 70% of the sequence windows had 70% secondary structure similarity, the cluster was ranked as average cluster. If no more than 30% of the sequence windows shared more than 70% secondary structure similarity, the cluster was ranked as a bad cluster.

For a given query sequence window, when a cluster had an average frequency profile closest to the profile of the given query, then that cluster's frequency profile was considered to be the best match of the given query sequence.

3.5 Experimental Results

Using the sliding window technique, we generated 6507 sequence windows (approximately %1 of the PISCES) to search for seeds from randomly selected proteins. These windows were removed from the database to prevent any bias when sequences were alike. We determined that this was a good proportion for searching for seeds because having more sequence windows would generate many matches in the database. For all our tests, these 6507 sequence and profile windows were used as the search queries. Seeds were selected by using the algorithms described above. These seeds were scanned against the 2290 protein sequences in the PISCES in order to find their best match out of 500,000 unique 9-mers (sequence window of size 9) in the PISCES database.

3.5.1. Seed selection results for the Sequence vs. Profile and Profile vs. Profile Algorithms

The results for the Sequence vs. Profile and the Profile vs. Profile methods are almost similar as can be seen in Fig. 3.3 and Fig. 3.4, respectively. In both of the methods, when the optimal alignment over the entire database was found, the probability of a significant structural similarity was low. This would correspond to the probability of a seed used by PHI-BLAST which was a structurally accurate homolog. It is clear that most of the seeds found have less than 70% structural similarity with their best match. These results indicate that the Sequence vs. Profile and the Profile vs. Profile methods cannot find seeds that would lead to a good sequence alignment.

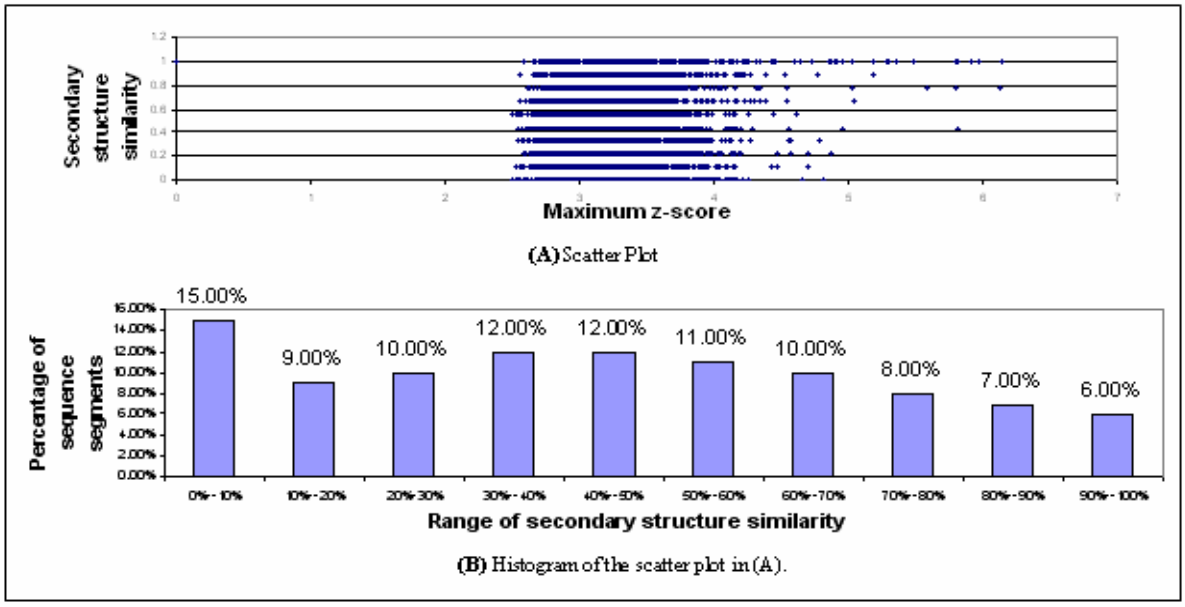


Figure 3.3 Sequence vs. Profile method results

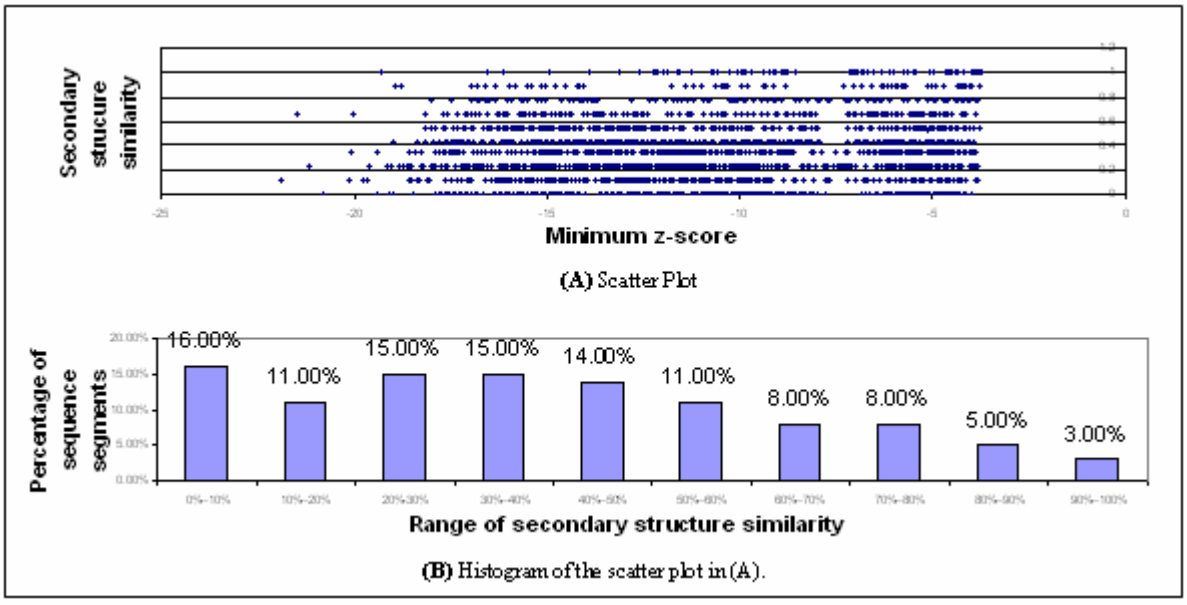


Figure 3.4 Profile vs. Profile method results

3.5.2. Dissimilarity search

The Sequence-Profile and Profile-Profile methods were also tested for their ability to find the most dissimilar structures in the database. We performed this test because we used an extreme value distribution measurement such as maximum and minimum z-scores in this work. The assumption is that the best matches found by the minimum and maximum z-scores of the sequence segments could correspond to most dissimilar structures as well. Searching for dissimilarity is important because it is possible that, if the structures of two proteins are dissimilar, then the words that form these structures are dissimilar.

All the given sequence segments are assigned a minimum z-score by using the Sequence-Profile method. The segments with minimum scores are compared with their best match in order to find the dissimilarity between them. The results are given in Figure 3.5, where each segment's minimum z-score and the secondary structure similarity with its best match are shown. The low secondary structure predictions correspond to most dissimilar structures. As can be seen from Figure 3.5 a, there is no relation between a segment's minimum z-score and its secondary structure similarity with its best match.

For the Profile-Profile method, all the given sequence segments are assigned a maximum z-score. The segments with maximum scores are compared with their best match in order to find the dissimilarity between them. The results are given in Figure 3.6, where each segment's maximum z-score and the secondary structure similarity with its best match are shown. The low secondary structure predictions correspond to most dissimilar structures. As can be seen from Figure 3.6a, there is no relation between a segment's maximum z-score and its secondary structure similarity with its best match.

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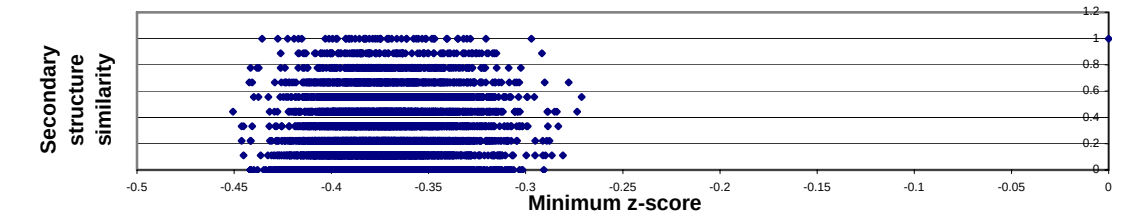
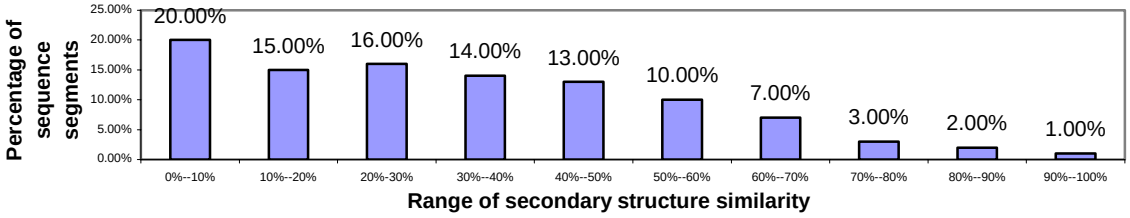


(A) Scatter Plot



(B) Histogram of the scatter plot in (A).

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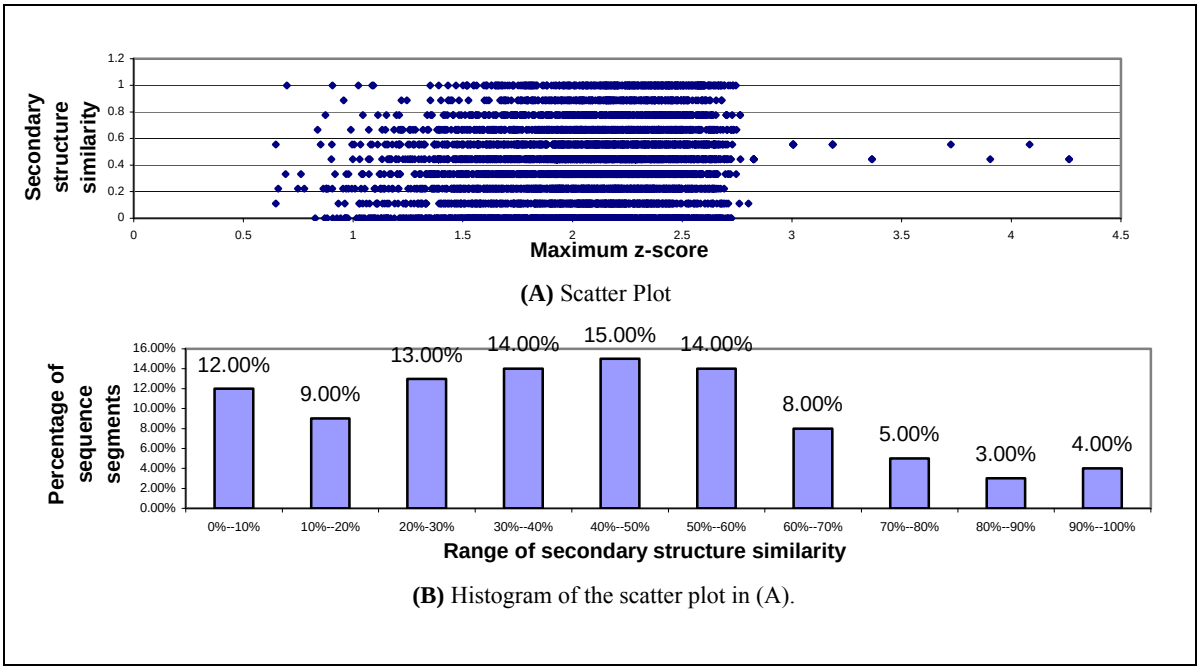
Figure 3.5 Structural dissimilarity for the most dissimilar sequences using Sequence-Profile Method.

Neither approach could accurately predict that two sequences have different structures because the best scores and worst scores of most sequence segments in the database have similar prediction accuracy.



(A) Scatter Plot

(B) Histogram of the scatter plot in (A).

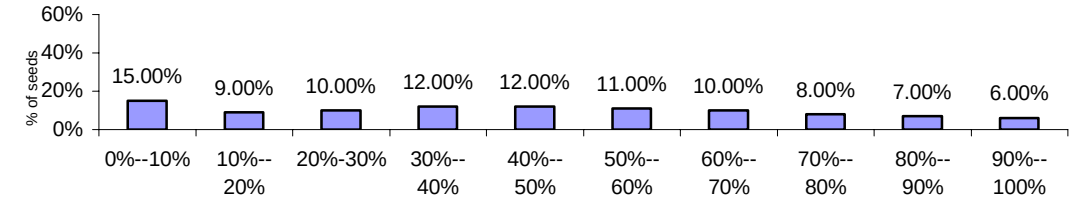
Figure 3.6 Structural dissimilarity for the most dissimilar sequences using Profile-Profile Method.

3.5.3. Profile vs. Clustered Profile seed selection results

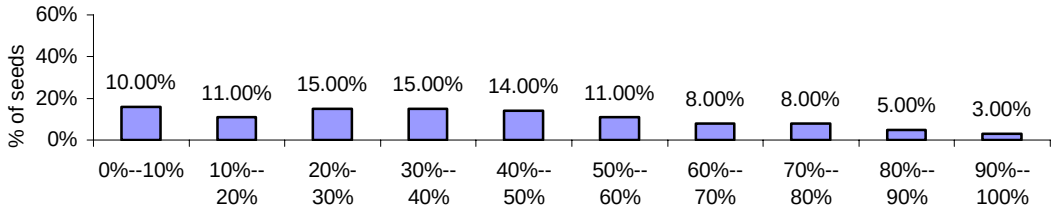
Neither the Sequence vs. Profile nor the Profile vs. Profile methods could select seeds that reflected local structural similarities. However, when the profiles are clustered prior to the search, significant structural similarity between the seeds and their best match are found when the Profile vs. Clustered Profile algorithm is used. Based on previous work, [75] we used 800 clusters and ranked each cluster as specified in the algorithm. Out of these 800 clusters, 345 clusters were ranked as high quality clusters and average quality clusters.

Figure 3.7(a) and 3.7(b) show the results for the Sequence vs. Profile and the Profile vs. Profile methods, respectively. Fig. 3.7(c) and Fig. 3.7(d) show that only 9% and 52% of sequence windows share above 70% structural similarity in bad sequence clusters and in average clusters, respectively.

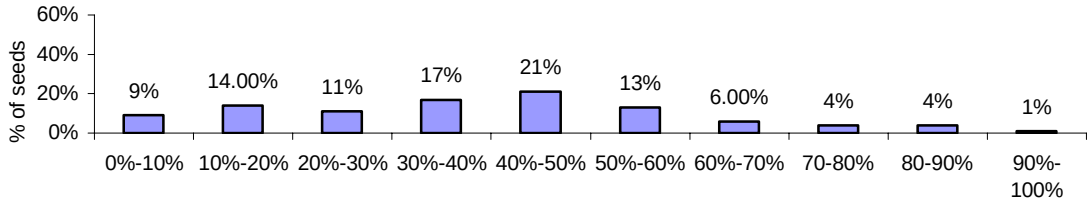
On the other hand, as can be seen from Fig. 3.7(e), high quality clusters were able to select sequence windows with very high structural similarity where 84% of sequence windows share above 70% structural similarity with the average cluster structure. These results show that the Profile vs. Clustered Profile algorithm can select seeds that have high structural similarity with the average cluster structure when high quality clusters are used.



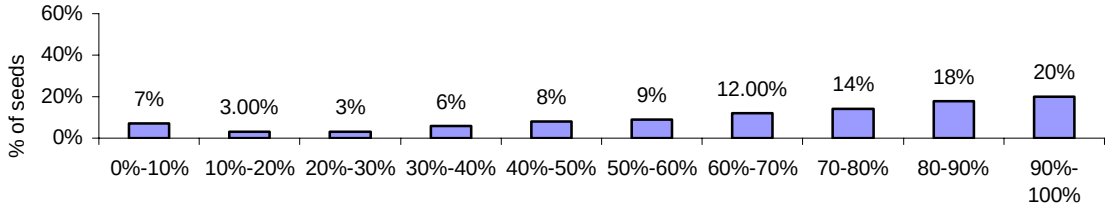
(a) Quality of seeds found in Sequence vs. Profile algorithm



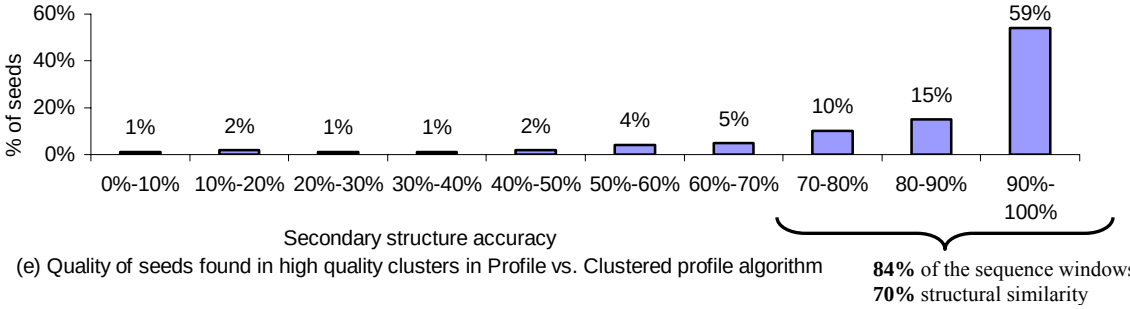
(b) Quality of seeds found in Profile vs. Profile algorithm



(c) Quality of seeds found in bad quality clusters in Profile vs. Clustered profile algotihm.



(d) Quality of seeds found in average quality clusters in Profile vs. Clustered profile algorithm



(e) Quality of seeds found in high quality clusters in Profile vs. Clustered profile algorithm

Figure 3.7 Seed selection results of all three algorithms

3.6 Conclusion

In this study, the factors involved in the accurate selection of seeds for protein sequence alignments were explored [4]. It is possible to identify seeds that are likely to share structural similarity with a meaningful *a priori* assessment of accuracy by using a profile-clustered profile approach. We used high order information identified by clustering and showed that it is reliable in small scales. We found that the look-up of these clustered sequence-based seeds for the best match works much better than the look-up of the individual frequency profile of each seed in the database. The predictive ability of these clusters suggests that there are distinct sequence-structure seeds. The dramatic improvement found by using high quality clustered profiles shows that higher order descriptions of sequence similarity are required for accurate results in the prediction of protein structure. This suggests that PHI-BLAST-like algorithms can be substantially improved if the database is clustered first. Our results show that when sequence windows are clustered and average profiles of these clusters are used for calculating similarity measure, it is possible to select seeds.

CHAPTER 4

Hybrid SVM Kernels for Protein Secondary Structure Prediction

The SVM model is a powerful methodology for solving problems in nonlinear classification, function estimation and density estimation. When the data are not linearly separable, they are mapped to a high dimensional future space using a nonlinear function, which can be computed through a positive definite kernel in the input space. Different kernel functions can change the prediction results remarkably. The goal of this work is to find the best kernel function that can be applied to different types of problems and application domains. We propose two hybrid kernels: SVM_{SM+RBF} and $SVM_{EDIT+RBF}$ [5]. SVM_{SM+RBF} is designed by combining the best performing radial basis function (RBF) kernel with a substitution matrix (SM)-based kernel developed by Vanschoenwinkel and Manderick [66]. $SVM_{EDIT+RBF}$ is designed by combining the edit kernel devised by Li and Jiang [46] with the RBF kernel. In our approach, two hybrid kernels are devised by combining the best performing RBF kernel both with the substitution matrix (SM)-based kernel [66] and with the edit kernel [46][68].

4.1. Hybrid kernel: SVM_{SM+RBF}

The SM-based kernel was developed by Vanschoenwinkel and Manderick [66]. The authors introduced a pseudo inner product (PI) between amino acid sequences based on the Blosum62 substitution matrix values [31]. PI is defined in [66] as follows:

Definition 1. Let M be a 20 × 20 symmetric substitution matrix with entries M(ai, aj) = mij where ai, aj are components of the 20-tuple A = (A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y) = (a1, . . . , a20). Then for two amino acid sequences x, x' $\in \sum$n with x = (ai1 , . . . , ain) and x' = (aj1 , . . . , ajn), with aik , ajk $\in$ A, i, j $\in$ {1, . . . , 20} and k = 1, . . . , n, their inner product is defined as:

$$< x \mid x' > \;=\; \sum_{k=1}^{n} M(a_{ik}, a_{jk}) \qquad (4.1)$$

Based on the PI above, the substitution matrix-based distance function between amino acid sequences is defined in [66] as follows:

Definition 2. Let x, x' $\in \sum$n be two amino acid sequences with x = (a_{i1} , . . . , a_{in}) and x' = (a_{j1} , . . . , a_{jn}) and let <x | x'> be the inner product as defined in equation (4.1) [66], then the substitution distance d_{sub} between x and x' is defined as:

$$d_{sub}(x, x') = \sqrt{< x \mid x > - 2 < x \mid x' > + < x' \mid x' >} \qquad (4.2)$$

Figure 4.1 shows how the rbf kernel is replaced with the substiturion kernel.

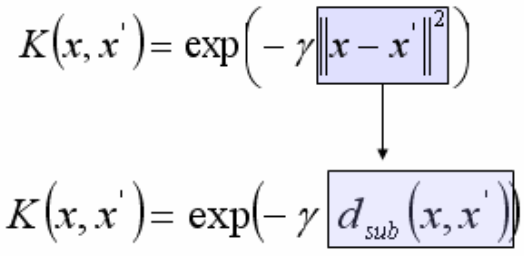


Figure 4.1 RBF Kernel vs. Substitution kernel

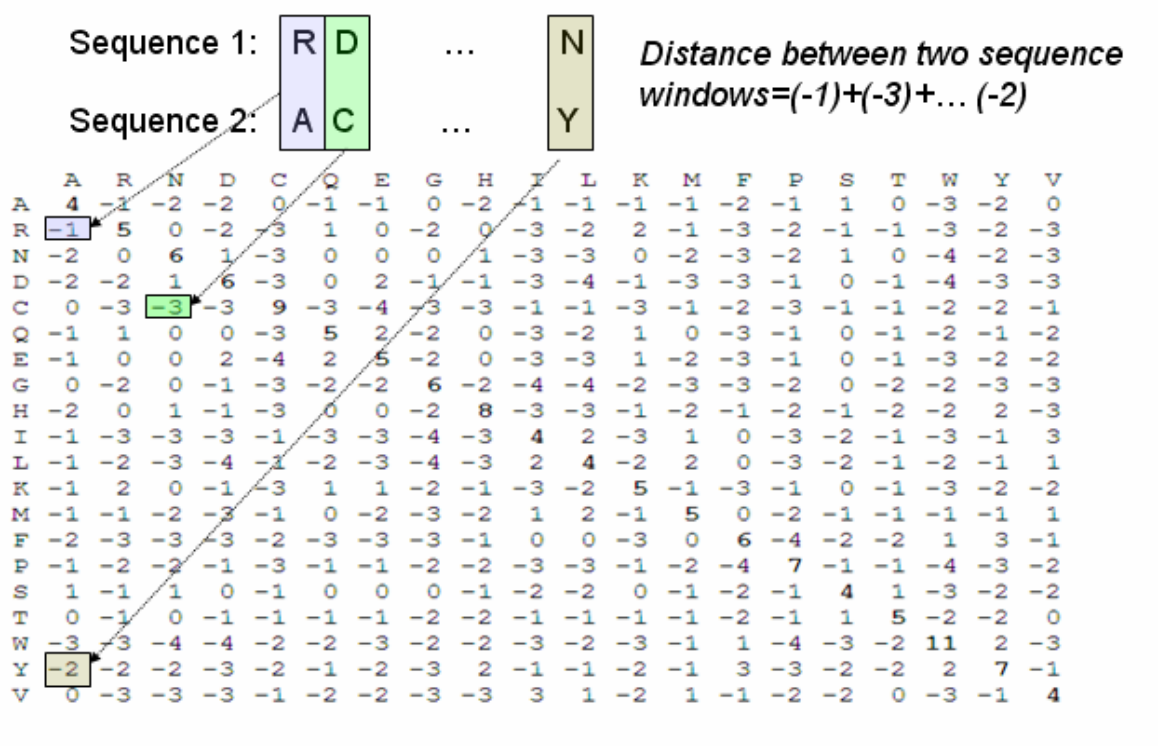


Figure 4.2 Distance between two sequence windows

In our approach, we combined the SM kernel with the RBF kernel. A diagram of the algorithm of SVM_{SM+RBF} is given in Fig. 4.3, which shows how a sequence segment is used in the hybrid kernel for finding distances with different kernel functions.

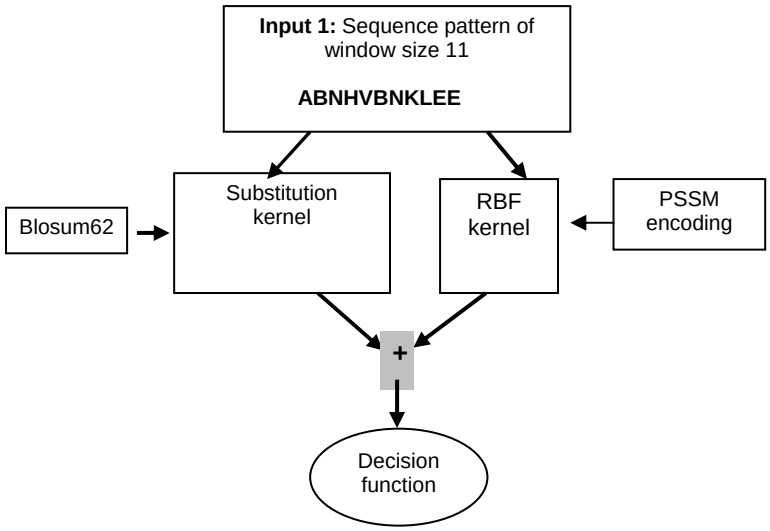


Figure 4.3 SVM_{SM+RBF} algorithm

The data encoding given to the SVM_{SM+RBF} is shown in detail in Figure 4.2. The data input for each sequence is the position-specific scoring matrix (PSSM) encoding of the sequence combined together with the sequence itself [37]. The same data encoding is used for $SVM_{EDIT+RBF}$.

4.2. Hybrid kernel: $SVM_{EDIT+RBF}$

The edit kernel was devised by Li and Jiang [47] to predict translation initiation sites in Eukaryotic mRNAs with SVM. It is based on the string edit distance, which contains biological and probabilistic information. The edit distance is the minimum number of edit operations (insertion, deletion, and substitution) that transform one sequence to the other. These edit operations can be considered as a series of evolutionary events. In nature, the evolutionary events happen with different probabilities. Li and Jiang [47] defined the edit

kernel as follows:

$$K(x, y) = e^{-\gamma \bullet edit(x, y)} \quad (4.3)$$

$$edit(x, y) = -\frac{1}{2}\left(\sum_i \log P(x_i \mid y_i) + \sum_i \log P(y_i \mid x_i)\right) \quad (4.4)$$

where the edit distance is the average of the negative log of the probability of mutating x into y and the negative log of the probability of mutating y into x. The authors modified the 1-PAM matrix to get the asymmetric substitution cost matrix (SCM) for the edit kernel above. In our approach, we combined the edit kernel with the RBF kernel. An example of $SVM_{EDIT+RBF}$ is given in Fig. 4.4, which shows how a sequence segment is used in the hybrid kernel for finding the distances.

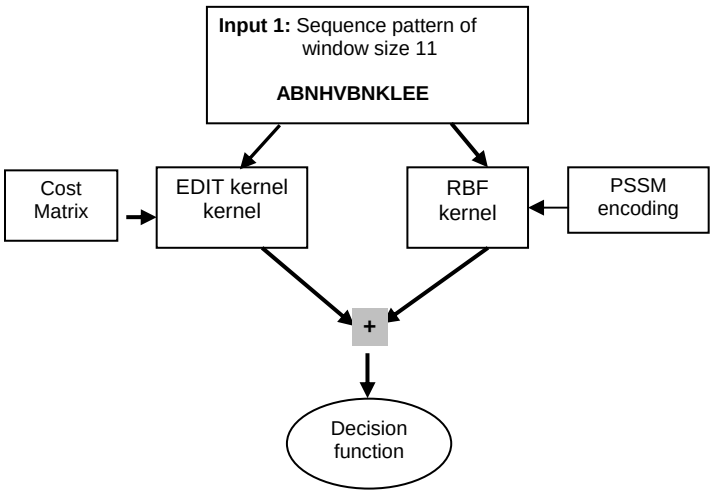


Figure 4.4 $SVM_{EDIT+RBF}$ algorithm

4.3. Experimental Results

The dataset used in this work includes 126 protein sequences obtained from Rost and Sander [59]. Sliding windows with eleven successive residues are generated from protein sequences. Each window is represented by a vector of 20x11. Twenty represents 20 amino acids and eleven represents each position of the sliding window. In Table 4.1, we show the results of the binary classifiers of the 6-fold cross-validation test for the protein secondary structure prediction. SVM_{freq} are from Hua and Sun [33] and the SVMpsi results are obtained by PSI-BLAST profiles from Kim and Park [41]. SVM_{RBF} is the profile which adopts the PSSM by Hu *et al.* [32]. As the result in [32] show, since PSSM encoding achieves the best results in the previous studies, we adopted the PSSM encoding scheme for the RBF kernel part of our hybrid kernel approaches.

Table 4.1 6-fold cross-validation of the binary classifiers

Binary Classifier	RS126		
	SVM**freq**	SVM**psi**	$SVM_{\mathbf{RBF}}$
H/~H	80.4	87.5	87.4
E/~E	81.3	86.3	86.8
C/~C	73.2	77.9	77.5
H/E	80.9	90.2	91.1
E/C	76.7	81.9	82.4
C/H	77.6	85.0	85.1

In Table 4.2, 6-fold cross-validation results of the binary classifiers obtained by using different kernels in SVM are shown. The hybrid SVM method SVM_{SM+RBF} proposed in this

work shows results that are almost identifcal to $SVM_{RBF.}$ This is because the data encoded for the RBF part in SVM_{SM+RBF} uses PSSM encoding, which is the same as in SVM_{RBF}. These results indicate combining SM with the RBF kernel cannot improve the accuracy the results where the RBF kernel is used alone. This means that the additional distance information from the SM part was not helpful in making the final decision. As alternatives, instead of adding the distance functions together, we have also tried different approaches, such as taking the maximum of the two distances returned by the two kernels, or giving different weight to each distance before sending it to the decision function. However, all these methods gave similar or worse results compared to those obtained by just adding the distance functions together. $SVM_{EDIT+RBF}$ could not achieve the results that SVM_{SM+RBF} achieved. This suggests that, for the protein secondary structure problem, SVM_{SM+RBF} is a more suitable kernel.

Table 4.2. 6-fold cross-validation of the binary classifiers

Binary Classifier	RS126				
	$SVM_{\mathbf{RBF}}$	$SVM_{\mathbf{SM}}$	$SVM_{\mathbf{EDIT}}$	$SVM_{\mathbf{SM+RBF}}$	$SVM_{\mathbf{EDIT+RBF}}$
H/~H	87.4	75.18	68.2	87.4	74.0
E/~E	88.2	78.44	40.0	86.8	76.7
C/~C	79.4	69.83	52.5	77.9	64.0
H/E	91.7	73.32	48.8	91.0	79.2
E/C	83.6	75.36	41.8	82.5	71.8
C/H	85.3	73.48	48.9	85.0	71.1

4.4. Conclusion

In chapter 4, we propose two hybrid kernels SVM_{SM+RBF} and $SVM_{EDIT+RBF}$. We tested these two hybrid kernels on one of the most widely studied problems in bioinformatics -the protein secondary structure prediction problem. For the protein secondary structure problem, our results achieved 91% accuracy in predicting the H/E binary classifier. In this case, the information in the substitution matrix reinforces the information in the RBF-on-PSSM profiles. However, this is not true with the edit distance. These results show that the data are consistent when the substitution matrix is used, but are not consistent when the edit distance is used. The edit distance kernel gives good results in [47], but not when used with our dataset in this work. Our results show that it is critically important to use mutually consistent data when merging different distance measures in support vector machines.

CHAPTER 5

A Feature Selection Algorithm based on Graph Theory and Random Forests for Protein Secondary Structure Prediction

In this work, we propose an algorithm that uses a graph-theory approach for feature selection. First, we apply this algorithm to the BLOSUM62 matrix; and then, based on the feature set produced by the algorithm, we use this feature set for condensing the PSSM matrix. This work attempted to reduce the feature space of the dataset using a graph-theoretical approach. Even though graph theory concepts have been around for more than a century, its concepts are just newly being explored for biological applications [12][66]. The clique search algorithm was applied to find all the cliques with different threshold values. We used Niskanen's and Ostergard's original implementation of *Cliquer* version 1.1 [49]. The code *Cliquer* is a set of C routines for finding cliques in an arbitrary weighted graph. It uses an exact branch-and-bound algorithm recently developed by Östergård [50]. Next, based on the newly designed algorithm, final cliques were determined. By merging the vertices within the same clique into one, the original feature space is reduced. Finally, this reduced feature set was applied to random forests and the performance was compared with the unreduced counterpart. In Fig. 5.1, the whole picture of this model is presented.

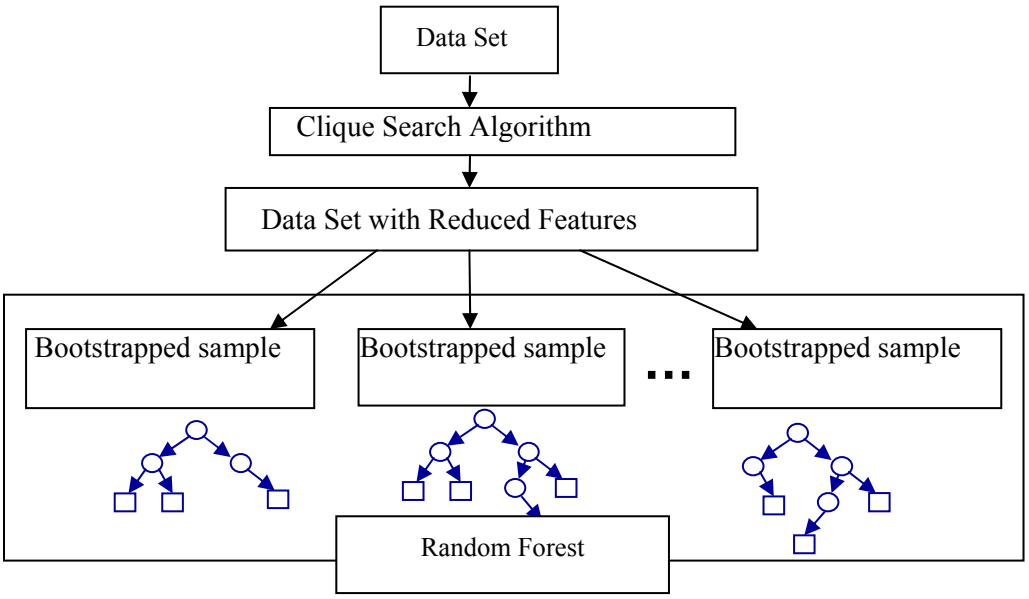


Figure 5.1 New model for protein secondary structure prediction

5.1. Encoding Schemes of the Data

Two matrices such as Blosum62 and PSSM were applied alone or combined with a feature reduction scheme. The BLOSUM62 matrix is a measure of differences between two distantly related proteins. The values in the BLOSUM62 matrix represent the possibility that two given amino acids will interchange with each other in the evolutionary process. The position-specific scoring matrix (PSSM) generated by PSI-BLAST; uses position-specific scores for each position in the alignment. Highly conserved positions have high scores and weakly conserved positions have low scores close to zero. Since each of these coding schemes captures different aspects of the properties of the amino acids, the combinations of these two different encodings would be more informative.

The above encoding profiles were generated based on the sliding window scheme. In the sliding window scheme, a window becomes one training pattern for predicting the structure of the residue at the center of the window. The optimal window size of the sliding window scheme was set as 13 based on previous research [32]. To reduce the noise in the training data and to minimize the memory requirement for training, the feature set was reduced based on the clique search algorithm. This approach is described in detail in the next section.

5.2. Feature Reduction Based on Cliques

A clique in an undirected graph G is a set of vertices V such that, for every two vertices in V, there exists an edge connecting the two. The subgraph induced by V is a complete graph. The size of a clique is the number of vertices it contains. The maximum clique problem is to find the largest clique in a given graph.

The BLOSUM62 matrix used in this study can be represented as a graph which consists of 20 different vertices. The edges among these 20 vertices can be introduced by applying different threshold values to the BLOSUM62 matrix. This study attempted to reduce the feature size by obtaining the cliques which occur commonly in different threshold values and by merging the vertices within the same clique. This process can be divided into the following three steps. The first step is converting the matrix into the adjacency matrix based on different threshold values ranging from -2 to 2. Each cell of the adjacency matrix has a value ‘1’ if there is an edge between two vertices or a value ‘0’ if there is no edge between them based on different threshold values. The second step is applying the clique search algorithm to each of these adjacency matrices. The third step is scanning through all the

cliques obtained from each matrix and finding the common cliques. The cliques of size 2, 3 or 4 vertices (n-mer) which share at least one physico-chemical property (polarity, hydrophobicity, or aromaticity, etc.) were considered for final decision. The common cliques were determined by counting the same vertices (n-mer) in each clique. Based on this algorithm, three most commonly occurring n-mers were found. These were merged into one-mers as follows:

- Q E → E
- I L M → L
- H F Y → Y

The pseudocode of this algorithm is given in Fig. 5.2 The physico-chemical property sets P in the pseudocode are described in Table 5.1.

```
Input: Blosum62 matrix B
    Threshold set T  T = {-2, -1, 0, 1, 2}
    Physico-chemical property sets P  P= {P1, P2, …, P8, P9}
Output: Common_Clique_Set C
Process:
    FOR each threshold i of T
        Adj_Matrix^i = Create_adjacency_matrix (B)
  END FOR
  FOR each adjacency matrix Adj_Matrix^i
            Clique_Set^i = Find_all_cliques (Adj_Matrix^i )
  END FOR
  FOR each clique set Clique_Set^i
      FOR each clique j  j ∈ Clique_Set^i
        if size_of(j) equals to 2 or 3 or 4
              FOR each Pi ∈ P
                if j ⊆ Pi
                      count++
            END FOR
                        Save the count into count_array
          END FOR
     END FOR
Common_Clique_Set C = Vote_and_Find_Top_Three(count_array)
```

Figure 5.2 Common clique search algorithm

Table 5.1 Physico-chemical property set

Set P	Physico-chemical properties	Amino acids in each set
P_1	Small	A, C, D, G, N, P, S, T, V
P_2	Hydrophobic	A, C, F, G, H, I, K, L, M, T, V, W, Y
P_3	Polar	C, D, E, H, K, N, Q, R, S, T, W, Y
P_4	Tiny	A, C, G, S
P_5	Aliphatic	I, L, V
P_6	Aromatic	F, W, Y
P_7	Charged	D, E, H, K, R
P_8	Positive	H, K, R
P_9	Negative	D, E

The BLOSUM62 matrix is reduced to the size of 15x15 based on the above compression. By applying the same reduction, the dimensions of the PSSM can also be compressed to Lx15. Here, L is the sequence length of the protein.

We also tested all other possible clique sizes between 1-20 in order to choose an optimal clique size. The test results are given in tables 5.2, 5.3 and 5.4. The highest accuracy was achieved when a clique size of 5 is used. These results indicate that the output of the algorithm already gives the optimal clique size which is 5.

Table 5.2 Finding optimal clique size (results between size 3-10)

Binary classifiers	Clique sizes							
	3	4	5	6	7	8	9	10
H/~H	87.3	86.2	93.6	86.8	87.0	85.9	83.9	75.1

Table 5.3 Finding optimal clique size (results between size 11-18)

Binary classifiers	Clique sizes							
	11	12	13	14	15	16	17	18
H/~H	74.2	68.8	67.3	67.3	68.0	64.1	63.5	66.6

Table 5.4 Finding optimal clique size (results between size 19-20)

Binary classifiers	Clique sizes		
	19	20	
H/~H	67.7	65.9	

5.3. Training and Testing

The commonly used RS126 set was applied to compare our results with previous studies. The RS126 data set was proposed by Rost and Sander and is known to be a non-homologous set which shares less than 25% sequence identity [59]. The random forests algorithm performs a bootstrap test with the training data. In other words, one third of the instances are

left out in the construction of the k^{th} tree; are applied for classification. Therefore, in random forests, we do not need to perform a cross-validation. Nor do we need to save a separate test set to obtain unbiased accuracy values. However, the current study applied two thirds of the original data for training and one third for testing to confirm the results obtained from the training data.

5.4.Parameter Optimization

In the random forests program, the only parameter which is optimized is the number of features, called $m_{try,}$ that are randomly selected at each node [15]. As a rule of thumb, the author suggested that it could be set to the square root of the number of whole features. Including this value, this study tested 4 different m_{try} values to find the optimum value.

5.5. Binary Classifiers

Six binary classifiers, such as three one-versus-rest classifiers (H/~H, E/~E and C/~C), and three one-versus-one classifiers (H/E, E/C and C/H) were created based on the previous study [32]. Here, the name ‘one’ in the one-versus-rest classifier refers to a positive class and the name ‘rest’ means a negative class. In the term one-versus-one classifier, the former “one” refers to a positive class and the latter “one” to a negative class. For example, the classifier H/~H classifies the testing sample as helix or not helix and the classifier E/C classifies the testing sample as sheet or coil. This paragraph is unclear. You introduce the one-versus-rest and one-versus-one notations, but then your example illustrates a different nomenclature: V and ~V.

5.6. Results

5.6.1. Parameter Optimization

Table 5.5 presents the result of applying different m_{try} values (the number of features randomly selected) based on the Blosum62 and the reduced PSSM concatenated encoding scheme. In the second column of the table, the value 22 is obtained from the approximate square root of the whole dimension of the feature: the whole dimension is (20+15) * 13 = 455. As can be seen from the table, the accuracy values are almost same even though we chose the larger m_{try} values. This means that the square root value is almost the optimal value.

Table 5.5 Comparison of different m_{try} values

Binary classifier	**Accuracy (%) for different m_{try} values**			
	22	**50**	**100**	**200**
H/~H	82.2 85.1	82.1 85.6	83.3 85.6	82.1 85.1

5.6.2. Encoding Scheme Optimization

Table 5.6 shows the result obtained by applying different encoding schemes to the random forests. Two different accuracy values are displayed. The first row is obtained by doing a bootstrap test on the training data and the second row by using the test data. As can be observed from the table, both the reduced Blosum62 matrix and the reduced PSSM

encodings present equal level of accuracy values when compared with the unreduced counterparts whether applied alone or applied in a concatenated form. This result proves that there is no information loss from the feature reduction and that our algorithm for this reduction works properly. Among all the different encoding schemes, the reduced PSSM encoding shows the best performance. The reduced PSSM encoding performs similarly to the concatenated encoding of the reduced PSSM and the BLOSUM62 matrix. The reduced PSSM shown in the last column has 13*15=195 features whereas the unreduced PSSM 13*20=260 features. This means that an approximate 25% feature reduction is achieved by using our algorithm while still achieving high accuracy.

Table 5.6 Comparison of different encoding schemes for H/~H

	PSSM	**Reduced PSSM**	**BLOSUM**	**Reduced BLOSUM**	**PSSM+ BLOSUM**	**Reduced PSSM+ BLOSUM**	**Reduced PSSM+ Reduced BLOSUM**
H/~H	82.3 85.5	82.5 85.7	76.9 80.7	77.2 80.8	82.3 85.1	82.2 85.1	81.7 85.0

In Table 5.7, all six binary classifiers are tested based on the BLOSUM and PSSM combined encodings. Once again, it can be observed that the reduced PSSM encoding has almost the same performance as the unreduced counterpart against all six binary classifiers.

Table 5.7 Accuracy results with BLOSUM+PSSM encoding

Binary classifiers	Accuracy for PSSM+BLOSUM	Accuracy for reduced PSSM+BLOSUM	Accuracy for reduced PSSM
H/~H	82.3 85.1	82.2 85.1	82.5 85.7
E/~E	83.9 81.1	83.7 81.1	84.0 81.0
C/~C	76.1 75.5	75.7 74.9	76.3 75.6
H/E	85.2 83.1	85.3 82.7	86.5 84.0
E/C	79.5 78.7	78.9 78.6	80.6 80.3
C/H	82.0 83.2	82.1 82.9	82.2 83.3

5.6.3. Time comparison

Table 5.8 shows the execution times of the reduced PSSM encoding scheme versus the PSSM+BLOSUM encoding scheme with different number of trees. Our proposed encoding scheme using reduced PSSM has a faster execution time. Also, when using 2000 trees, PSSM+BLOSUM encoding scheme did not run after a few hours due to its high dimensionality whereas reduced the PSSM encoding could run. These results show that the reduced PSSM encoding could be used to reduce the space and time complexity drastically where the data dimensionality is very high.

Table 5.8 Comparison of execution times for reduced PSSM vs. PSSM+BLOSUM

Tree size	Encoding Scheme	
	PSSM+BLOSUM	**Reduced PSSM**
100	25min 58.9s	5min 53.7s
500	153min 50.6s	31min 8.5s
1000	267min 31.9s	66min 15.8s
2000	_	124min 24.7s

5.6.4. Random forest vs. SVM

We have proposed an initial new model that uses support vector machines and cliques for feature selection, and some initial results have been obtained for the protein secondary structure prediction problem. This model is shown in Figure 5.3.

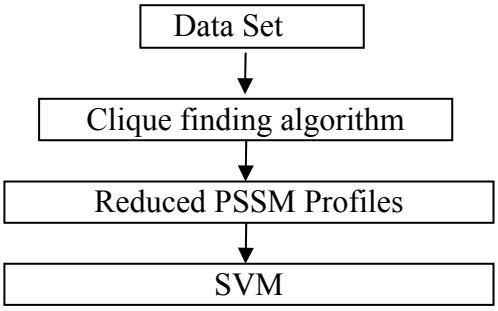


Figure 5.3 Prediction Model

The reduced feature set was applied to support vector machines and the performance was compared both with the unreduced counterpart and with the random forests method for protein secondary structure prediction. The results are presented in Table 5.9.

Table 5.9 Random forest vs. SVM comparison for different encoding schemes

Binary classifiers	**Random forest**		**SVM**	
	Accuracy for PSSM+BLOSUM	Accuracy for reduced PSSM	Accuracy for PSSM+BLOSUM	Accuracy for reduced PSSM
H/~H	85.1	85.7	**92.8**	**93.6**
E/~E	81.1	81.0	**83.3**	**87.1**
C/~C	75.5	75.6	**72.4**	**77.6**
H/E	83.1	84.0	**88.2**	**90.8**
E/C	78.7	80.3	**79.8**	**82.4**
C/H	83.2	83.3	**83.9**	**84.5**

As can be seen from the table, SVM produces much better accuracy than the random forests which improved our previous accuracy results.

5.7. Conclusion

In this work, we proposed a novel algorithm for feature selection based on cliques and evolutionary information of proteins. We tested our algorithm using random forests and different encoding schemes for the secondary structure problem in proteins. These algorithms were tested on both condensed and non-condensed data sets. We found out that the prediction accuracies for both data sets were similar. These results show that a significant amount of space and time can be saved while still achieving the same high accuracy results by using a subset of the features when these features are carefully selected.

These results show that it is important to select features from the data that are more significant for training and testing instead of using the entire feature set. Also, using our novel algorithm, we achieved an approximate 25% reduction in space and time. We tested

our algorithm using SVM as a machine learning method instead of random forests and achieved high accuracy. Finally, we propose that, as a subject for further research, SVM can be used instead of random forests in order to increase prediction accuracy.

CHAPTER 6

New Binary Classifiers for Protein Structural Boundary Prediction

Proteins are primarily made up of amino acids which determine the structure of a protein. Protein structure has three states called primary structure, secondary and tertiary structure. The primary structure of the protein is its amino acid sequence. The secondary structure of a protein is formed from recurring shapes called the alpha-helix, the beta sheet, and the coil. The tertiary structure of the protein is the spatial assembly of helices and sheets and the pattern of interactions between them. Predicting the secondary and tertiary structure of proteins from their amino acid sequences is an important problem; knowing the structure of a protein aids in understanding how the functions of proteins in metabolic pathways map for whole genomes, in deducing evolutionary relationships, and in facilitating drug design.

It is strongly believed that protein secondary structure delimits the overall topology of the proteins [26] Therefore, during the past 25 years, many researchers have tried to understand how to predict the secondary structure of a protein from its amino acid sequence. Many algorithms and machine learning methods have been proposed for this problem [2][6] [40][42]. The algorithms for predicting secondary structure of proteins have reached a plateau of roughly 90%. Much more success has occurred with motifs and profiles [16].

The common approach to solve the secondary structure prediction problem has been to develop tools that predict the secondary structure for each and every amino acid (residue) of a given protein sequence. In this work, we propose new binary classifiers which do not

require the correct prediction of each and every residue in a given protein segment. The new binary classifiers predict only the start or end of a helix, sheet or coil. In figure 6.1, this concept is illustrated. Fig 6.1 represents the tertiary structure of a protein with its secondary structure regions colored in different shades. The point where one secondary structure element ends and another one begins is called a "structural transition" throughout this chapter.

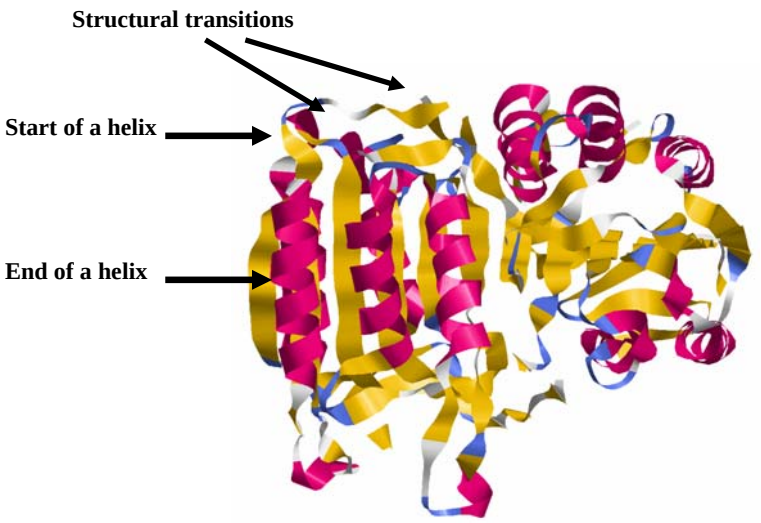


Figure 6.1 Structural transitions of a protein

Protein sequences may have specific residue preferences at the end or start of secondary structure segments. For example, it has been shown that specific residue preferences exist at the end of helices, which is called helix capping. Recent research has suggested that it is possible to detect helix-capping motifs [7]. However, these results reflect a linear decision function based on amino acid frequencies. It is well known that non-linear decision functions, for example those implemented with the Support Vector Machines (SVM), dramatically outperform linear decision functions when the underlying data are nonlinear [68]. In this work, we use a machine learning approach based on SVM to predict the helix

capping regions of a given protein sequence. These helix capping regions indicate where a helix ends. The same method is also used for predicting the starting points of helices and to predict the end and starting points of coils and sheets. The end and starting points of secondary structures are also called structural transition boundaries.

6.1. Problem Formulation

In this study, we adopted the most generally used DSSP secondary-structure-assignment scheme [39]. The DSSP classifies the secondary structure into eight different classes: H (α-helix), G (310-helix), I (π-helix), E (β-strand), B (isolated β-bridge), T (turn), S (bend), and - (rest). These eight classes were reduced into three regular classes based on the following method: H, G and I were reduced to H; E to E; and all others to C.

6.1.1. Traditional problem formulation for the secondary structure prediction

The traditional problem formulation is stated as:

Given a protein sequence $a_1a_2\ldots a_N$, find the state of each amino acid a_i as being either:

- H (helix) or
- E (beta strand) or
- C (coil).

The quality of the secondary structure prediction is measured with a "3-state accuracy" score called Q_3. Q_3 is the percent of residues that match reality. Most of the previous research adopted Q_3 as an accuracy measurement.

6.1.2. New problem formulation for the transition boundary prediction

The new problem formulation is stated as follows:

Given a protein sequence profile, find the state of each amino acid ai as being either:

- The start of a H (helix), E (beta strand), or C (coil) or
- The end of a H (helix), E (beta strand), or C or
- Neither of the above (named as 'X': doesn't matter)

Here, we used a new scoring scheme that we call Q_T ($T_{ransition}$) which is similar to Q_3. Q_T is the percent of residues that match reality. We had to change the scoring scheme to Q_T because Q_3 scoring scheme takes into account all the residues whereas Q_T takes into account only the residues that are necessary for prediction.

$$Q_T = \frac{\sum_{i \in \{H,E,C\}} \#\ of\ \ correctly\ \ \ predicted\ \ \ transition\ \ \ residues_{\ i}}{\sum_{i \in \{H,E,C\}} \#\ of\ \ transition\ \ residues\ \ \ i} \quad (6.1)$$

In Q_T scoring scheme the number of correctly predicted transition residues of class H, E, o C are divided by the number of all transition residues of class H, E or C.

6.2. Method

6.2.1. Motivation

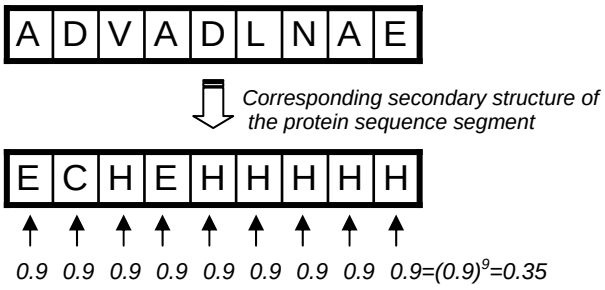


Figure 6.2 A 9-mer with helix junction

Given a protein sequence of a 9-mer, let the middle element of this 9-mer be the starting position of a helix as it shown in Figure 6.2. Our goal is to determine whether the middle residue is the start or end of a helix. If we use the traditional binary classifiers (such as H/~H), first we must correctly identify all the residues in the whole segment. We need to correctly predict 3 consecutive residues as H (at least 4 residues are needed for a helix) and the rest of the residues should be ~H. In this case, we have to make 9 predictions, and ideally we should be correct all 9 times. However, the probability that we can predict all 9 residues correctly in the protein segment is at maximum .35 if we assume that our chance of making each prediction correctly is 0.9 and that this probability of success is independent of the other predictions.

In the next section, we explore how to overcome the problem of making 9 predictions for a given 9-mer and how to reduce it to a problem of making only one prediction per 9-mer.

6.2.2. A new encoding scheme for the prediction of starts of H, E and C.

The goal of our new encoding scheme is shown in Fig 6.3 where a new binary classifier has to make only one guess instead of 9 guesses. Here, the new encoding scheme for representing the starting points of helices is shown as an example. The same encoding is applied to both sheets and coils.

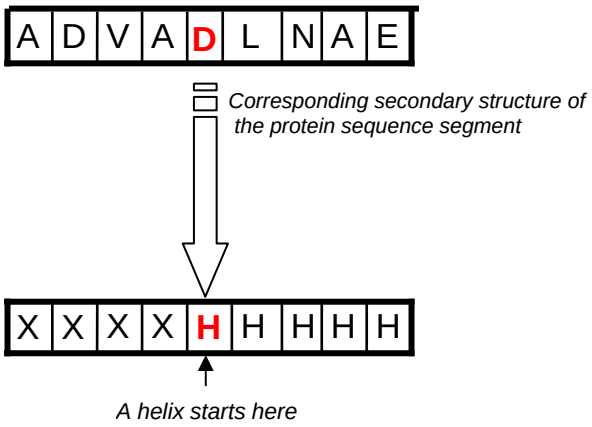


Figure 6.3 New encoding scheme for Helix start

In Figure 6.3, the illustration of the new encoding scheme is presented.

The rules of the new encoding scheme are as follows:

In order for the middle residue to be classified as the start of a helix, the conditions are:

1. The residues corresponding to X's can be C, H or E, but no two consecutive H's are allowed.
2. The secondary structure of the middle residue must be H.
3. All residues after the middle residue must be H.

If all three rules are satisfied, the protein segment is represented by the new encoding as the start of a helix (H_{start}). If not, the protein segment is represented as ~H_{start} (not the start of a helix).

6.2.3. A new encoding scheme for the prediction of ends of H, E and C.

Similar to the the method in section 6.2.2 the new encoding scheme for representing the ends of helices is shown as an example is shown in Fig 6.5. The same encoding is applied to sheets and coils.

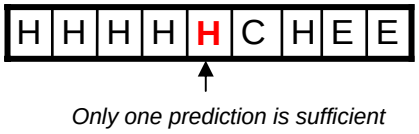


Figure 6.4 A 9-mer with helix end

In the new encoding scheme, the protein sequences are classified as the following:

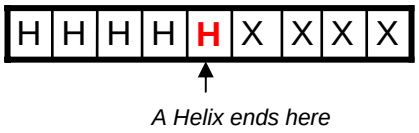


Figure 6.5 New encoding scheme for Helix start

The rules of the new encoding scheme are as follows. These are similar to the rules in section 6.2.2, however used for predicting the ends of secondary structures:

In order for the middle residue to be classified as a helix end, the conditions are:

1. The residues corresponding to X's can be C, H or E, but no two consecutive H's are allowed.
2. The secondary structure of the middle residue must be H.
3. All residues before the middle residue must be H.

If all three rules are satisfied, the protein segment is represented by the new encoding as the end of a helix (H_{end}). If not, the protein segment is represented as $\sim H_{end}$ (not the end of an helix).

6.3. New binary classifiers

In the traditional secondary structure prediction approach, usually six binary classifiers, such as three one-versus-rest classifiers (H/~H, E/~E and C/~C) and three one-versus-one classifiers (H/E, E/C and C/H) are used. Here, the name 'one' in one-versus-rest classifier refers to a positive class and the name 'rest' means a negative class. Likewise, the name 'one's in one-versus-one classifier refers to positive class and negative class respectively. For example, the classifier H/~H classifies the testing sample as helix or not helix and the classifier E/C classifies the testing sample as sheet or coil.

The six new binary classifiers that are proposed are the following:

Binary Classifier 1:

H_{start}/~H_{start}: This binary classifier classifies the positive samples as the start of a helix and negative samples as not being the start of a helix.

Binary Classifier 2:

E_{start}/~E_{start}: This binary classifier classifies the positive samples as the start of a sheet and negative samples as not being the start of a sheet.

Binary Classifier 3:

C_{start}/~C_{start}: This binary classifier classifies the positive samples as the start of a coil and negative samples as not being the start of a coil.

Binary Classifier 4:

H_{end}/~H_{end}: This binary classifier classifies the positive samples as the end of a helix and negative samples as not being the end of a helix.

Binary Classifier 5:

E_{end}/~E_{end}: This binary classifier classifies the positive samples as the end of a sheet and negative samples as not being the end of a sheet.

Binary Classifier 6:

C_{end}/~C_{end}: This binary classifier classifies the positive samples as the end of a coil and negative samples as not being the end of a coil.

6.4. SVM kernel

We used a radial basis kernel (RBF) since it was optimal when used for secondary structure prediction:

$$K(x, y) = e^{-\gamma \| x - y \|^2} \qquad (6.2)$$

Here, x and y are two input vectors containing different feature values and γ is the radial basis kernel parameter. Radial basis kernels depend on a numerical representation of the input data.

6.5. Choosing the window size

In order to choose an optimal window size for the proposed encoding scheme for a given protein segment, we tried different window sizes on the smaller dataset RS126. We used the PSSM profiles of the dataset RS126 during the tests. As a prediction method, the SVM RBF kernel was used. Using the sliding window scheme, first each k-mer from a protein sequence is extracted. Each k-mer is classified as a positive or negative sample. If the middle residue satisfies the encoding scheme as described in section 6.2, it is marked as a positive sample: H_{start}, E_{start}, or C_{start}. Otherwise it is marked as a negative sample $\sim H_{start}$, $\sim E_{start}$, or $\sim C_{start}$,

Fig 6.6 shows the Q_T prediction accuracy results of all the six new binary classifiers used with the SVM RBF kernel and the RS126 dataset. The prediction accuracy of SVM varied for different window sizes. The best overall prediction accuracy was achieved when the window size was 9.

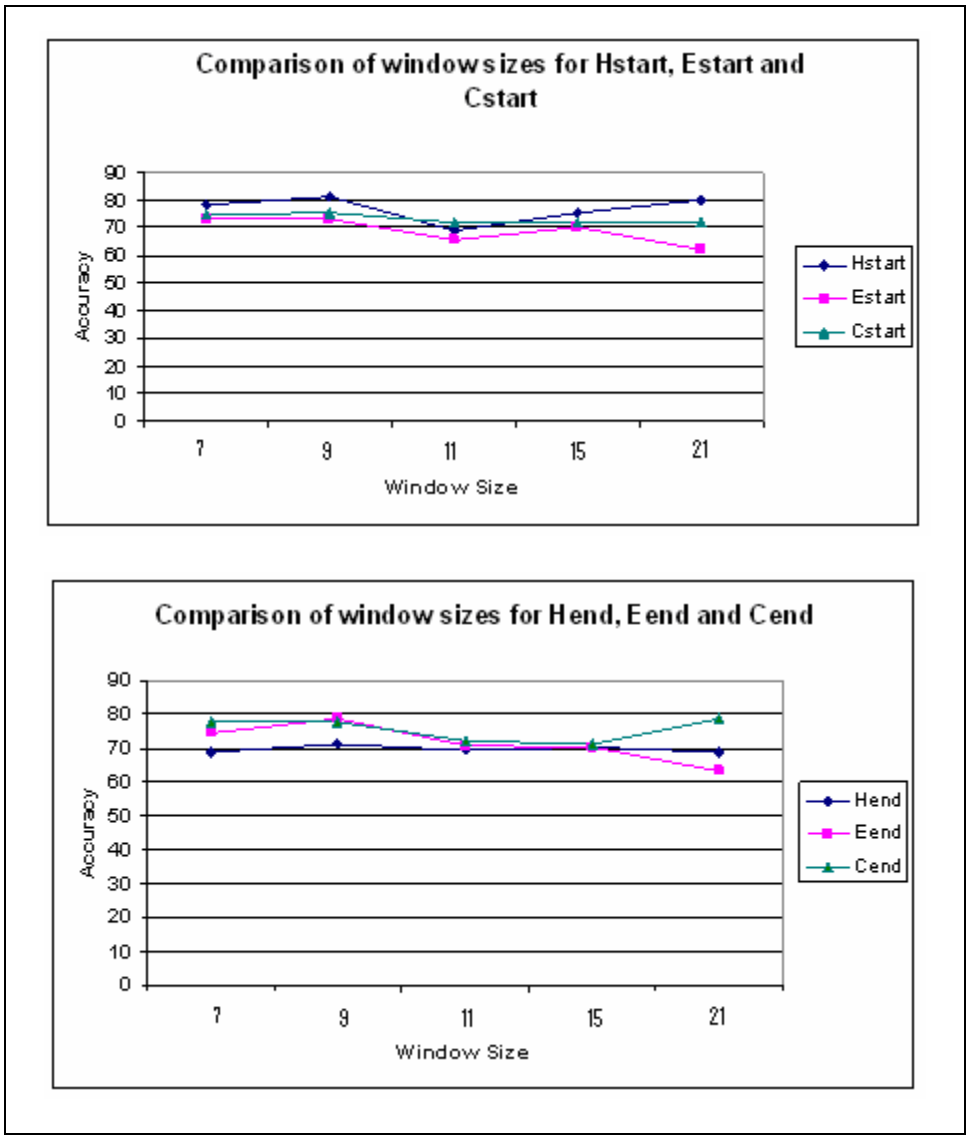


Figure 6.6 Accuracy of H_{end}, E_{end} and C_{end} binary classifiers for RS126 dataset

Fig 6.7 shows the Q_T prediction accuracy results of all the six new binary classifiers used with the SVM RBF kernel and the CB513 dataset. The prediction accuracy of SVM varied for different window sizes. The best overall prediction accuracy was achieved when the

window size was 9 for CB513 data which is similar to the results of RS126 data shown in Fig. 6.6.

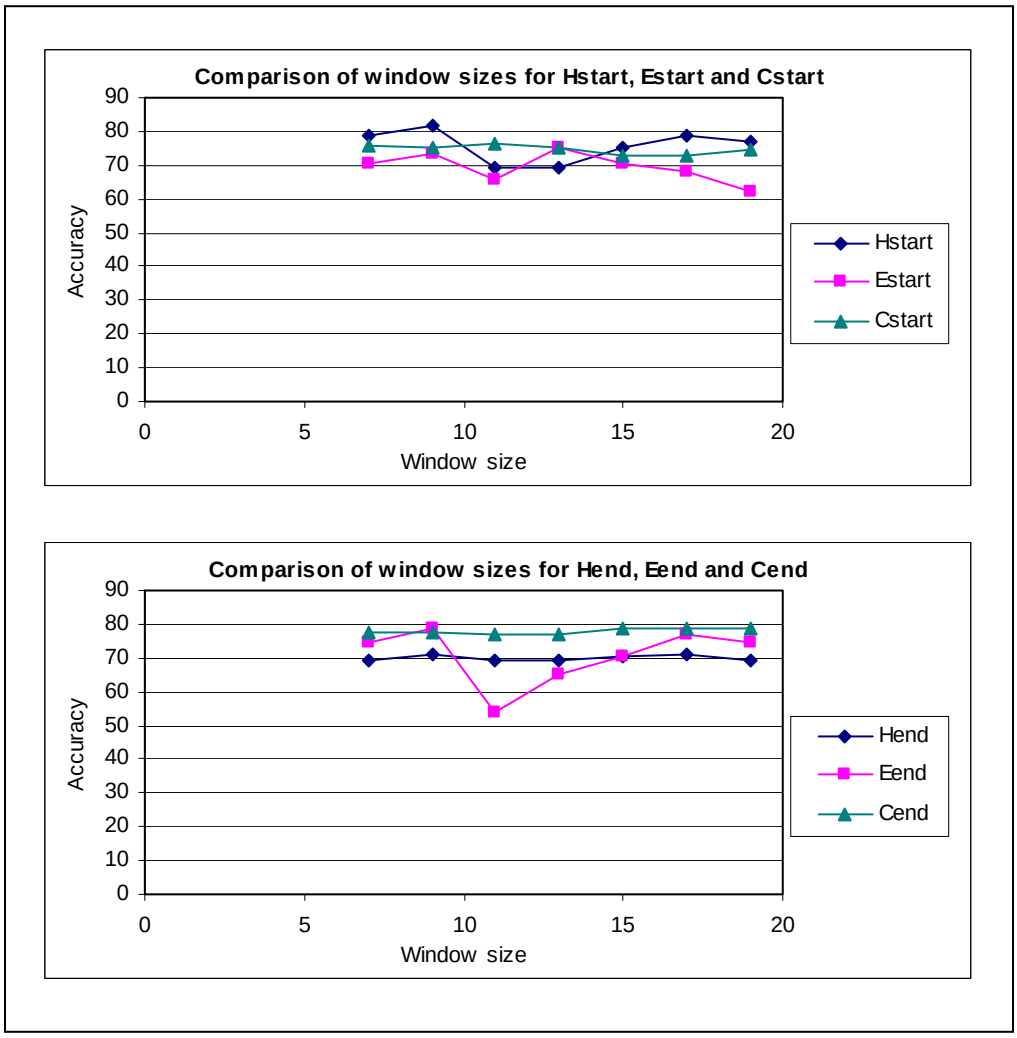


Figure 6.7 Accuracy of H_{end}, E_{end} and C_{end} binary classifiers for CB513 dataset

For the later experiments and for the larger dataset CB513 dataset a window size of 9 was used for testing the new binary classifiers.

6.6. Test results of the binary classifiers

Table 6.1 shows the Q_T prediction accuracy results of all six binary classifiers H_{start}/~H_{start}, E_{start}/~E_{start} , C_{start}/~C_{start} and H_{end}/~H_{end} , E_{end}/~E_{end} and C_{end}/~C_{end} used with the SVM RBF kernel with a window size of 9. We used the PSSM profiles of the dataset CB513 during these tests. Since there were many negative samples, we balanced the negative and positive samples in the dataset by randomly choosing from the negative samples for training the SVM. The results are given in Table 6.1. The probability of SVM correctly predicting the start of helices is 81.5%, which is much higher than the 35% theoretical bound for per-residue prediction. The probability of successfully predicting the end of a helix is also high--approximately 71.33%. This shows that there is more of a signal in the data indicating the start of helices than there is a stop signal. The start and end positions of strands and coils are predicted with approximately 75% accuracy.

These results show that, by training a classifier such as SVM to predict the secondary structure transition boundaries, it is possible to detect where helices, strands and coils begin and end with high accuracy. Furthermore, the detection of these secondary structure transition boundaries is performed on the basis of one prediction rather than trying to predict correctly all the residues in a given sequence segment, the probability of which would theoretically be only roughly 35%.

Table 6.1 Prediction accuracies of the new binary classifiers

Binary Classifier	Accuracy (TP+TN)/ (TP+TN+FN+FP)*	Recall (TP/TP+FN)	Specifity (TN/TN+FP)	Precision (TP/(TP+FP)
$H_{start}/\sim H_{start}$	81.5	78.5	84.16	83.33
$E_{start}/\sim E_{start}$	73.16	73.33	73.16	73.16
$C_{start}/\sim C_{start}$	75.33	78.33	72	74.33
$H_{end}/\sim H_{end}$	71.33	86.16	66.66	69.5
$E_{end}/\sim E_{end}$	78.66	82	75.33	77.66
$C_{end}/\sim C_{end}$	77.66	79	76	77.5

** TP: TRUE POSITIVE TN: TRUE NEGATIVE FP: FALSE POSITIVE FN: FALSE NEGATIVE*

6.7. Accuracy as a function of helix sizes

Fig 6.8 shows the comparison between the prediction accuracy levels of helix starting and end points as a function of the number of turns in the helix. One can see that the prediction accuracies of the binary classifiers $H_{start}/\sim H_{start}$ and $H_{end}/\sim H_{end}$ reach a maximum value when the helix has 2.25 turns. Since a helix has about 4 residues per turn, this corresponds to a window size of 9 residues. At different number of turns of a helix, the accuracies are lower. This also proves that choosing a window size of 9 residues is optimal for the transition boundary prediction problem.

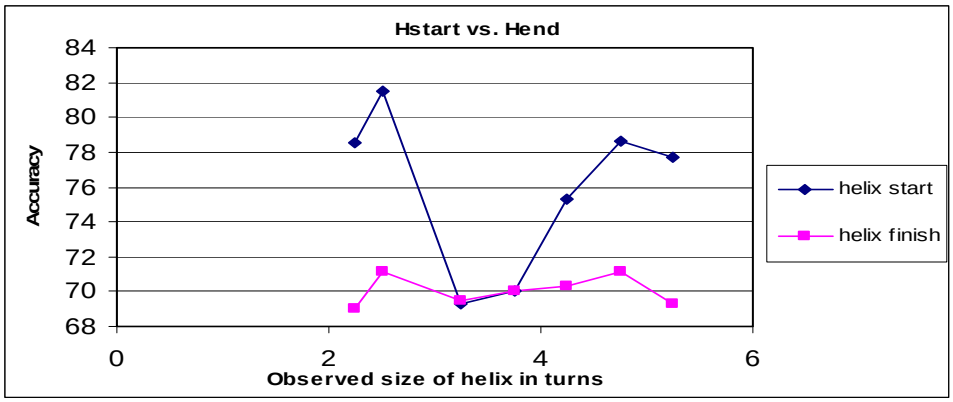


Figure 6.8 Accuracy levels of H_{start} and H_{end}

6.8. Comparison of traditional binary classifiers to the new binary classifiers

There are several studies that focus on finding where the structural segments start and end. Aydin *et al.* have shown that new dependency models and training methods bring further improvements to single-sequence protein secondary structure prediction [8]. Their results improve most Q_3 accuracy results by 2%, which shows that considering amino acid patterns at segment borders increases the prediction accuracy. Some other approaches are focused on finding the end of helices. The reason for this is that the helices (alpha-helices) are the most abundant regular secondary structure and that a certain residue preference exists at the ends of helices [71]. However, current secondary structure prediction programs can not identify the ends of helices correctly in most cases. The same rule applies to strands although the residue preferences for strand termini are not as strong as in helices. Wilson *et. al.* used cumulative pseudo-free energy calculations to predict helix start positions and achieved 38% prediction accuracy. We achieved around 80% Q_T accuracy using SVM, which is of course significantly higher.

One could question what our Q_3 overall prediction accuracy is. Most of the current secondary structure prediction methods try to solve the problem at a per-residue level, whereas we try to solve the prediction at a per-segment level. In this work, we proposed binary classifiers that target the prediction of the start and end positions of helices, strands and coils. Therefore, in order to be able to compare our prediction accuracy to the current prediction methods, we derived a method that converts our Q_T accuracy results to the standard Q_3 and vice versa.

6.8.1. Estimate of the Q_3 from Q_T and Q_T from Q_3

When, traditional binary classifiers such as one-versus-rest classifiers (H/~H, E/~E and C/~C), and one-versus-one classifiers (H/E, E/C and C/H) are used, their prediction accuracies are measured using a Q_3 measurement. In the Q_3 measurement, a prediction for each and every residue of a protein sequence is done. In order to determine whether a given protein sequence is the start or end of a secondary structure with the traditional binary classifiers, each residue's secondary structure must be predicted first. However, it is clear that, even with the 90% accuracy per residue, the probability of independently predicting k residues correctly is 0.9 to the k^{th} order. In order to calculate a Q_3 measurement of a given a protein sequence window (of size k), a prediction for each and every residue in that window must be made using the traditional binary classifiers. However, with the proposed new binary classifiers, only one prediction per window is enough to tell whether that window represents the end or start of a helix, sheet or coil. Besides, the overall prediction probability is slightly pessimistic because the estimates may not be fully independent.

Based on the above reasoning, in order to be able to compare our results to the traditional binary classifiers which calculate the prediction accuracy per residue, we derived a method using the following assumption. Given a protein segment of window size k, we assumed that the prediction of each residue in that window is truly independent of the other residues in that window. Then, we converted the traditional Q_3 accuracy measurement to our accuracy measurement Q_T, using the following equation:

$$Q_T = Q_3^{(\text{window size})} \quad (6.3)$$

The formula above basically states that, the fewer number of predictions made for a given protein window, the higher the chances are that the prediction is correct. Using the traditional binary classifiers, given a protein window of size k, k predictions must be made in order to see what that protein sequence segment is. Using the binary classifiers proposed in this work, only one prediction is enough. The inverse of the formula above is:

$$Q_3 = e^{\ln(Q_T)/k} \quad (6.4)$$

The inverse of the formula gives us the corresponding Q3 accuracy as a function of Q_T.

6.8.2. Traditional binary classifiers vs. new binary classifiers

In order to make a fair comparison, we took Q_3 measurements for the H/~H, E/~E and C/~C binary classifiers from [32][33] which are one of the highest Q_3 measurements for these binary classifiers, and estimated their Q_T measurements. We also converted the Q_T results in Table 6.1 to Q_3 measurements and listed the results in Table 6.2 and Table 6.3.

Table 6.2 Estimated Q_3 results

Binary classifiers	**Q_T converted to Q_3**	
	Q_T	Q_3
H/~H	83.17	96.31
E/~E	80.5	95.67
C/~C	76.5	94.65

Table 6.3 Estimated QT results

Binary classifiers	**Q_3 converted to Q_T**	
	Q_3*	Q_T
H/~H	87.18	50.35
E/~E	86.02	47.09
C/~C	77.47	36.01

*Q3 measurements from Hu et al, 2004 and Hua and Sun, 2001

Table 6.2 shows our Q_T accuracy calculations converted to the corresponding Q_3 accuracies. When Q_3 accuracies are converted to Q_T measurements as shown in table 6.3, the accuracies are low. (Note, these estimates are based on the assumption that each residue prediction is independent of all the others.) These results show that using the new binary classifiers gives higher prediction accuracy than the traditional binary classifiers. These results also prove that it is better to make predictions using a per-segment window rather than a making them per residue. In other words, we should split the data in big chunks (segments) and make predictions using these segments instead of trying to predict each and every piece of data (residues).

6.9. Test results on individual proteins outside the dataset

In order to prove that the new proposed encoding scheme works, we have run blind tests on individual proteins. The test results are given in Table 6.4. In all the test cases, the accuracy, recall, specifity and precision values are high as expected. However, the precision values are low. The reason for this is the unbalanced nature of the dataset. In our training datasets, we have many negative samples whereas the positive samples are roughly 1% of the number of the negative sets. This is a major problem with these kinds of datasets. The false positives (FPs) are high because we are dealing with 100 times more examples of negative cases than positive cases. These results imply that it is very hard to get a high precision due to the unbalanced nature of the datasets. The false positives overwhelm the correct matches. This is the truly difficult aspect of using minority classes. The good accuracy shows that there is a signal in the data that we can extract. However, because there are so many more negatives matches, we get large number FPs. What we discover by this analysis is that there is a signal that SVM selects because we have high accuracy; however, we can not get high precision values because there are very few examples of the minority classes. Therefore, we give he results for both balanced data and unbalanced data.

Table 6.4 Protein ID: CBG

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	0.77	0.62	0.77	0.13
$E_{start}/\sim E_{start}$	0.80	0.53	0.81	0.9
$C_{start}/\sim C_{start}$	0.71	0.59	0.72	0.17
$H_{end}/\sim H_{end}$	0.79	0.54	0.80	0.13
$E_{end}/\sim E_{end}$	0.81	0.59	0.82	0.11
$C_{end}/\sim C_{end}$	0.72	0.57	0.73	0.17

Table 6.5 Protein ID: CELB

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	0.89	0.2	0.99	0.03
$E_{start}/\sim E_{start}$	0.75	0.39	0.78	0.13
$C_{start}/\sim C_{start}$	0.58	0.27	0.62	0.07
$H_{end}/\sim H_{end}$	0.91	0.70	0.92	0.17
$E_{end}/\sim E_{end}$	0.79	0.42	0.82	0.17
$C_{end}/\sim C_{end}$	0.63	0.51	0.64	0.13

Table 6.6 Protein ID: BAM

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	0.79	0.57	0.79	0.09
$E_{start}/\sim E_{start}$	0.69	0.50	0.69	0.06
$C_{start}/\sim C_{start}$	0.66	0.75	0.65	0.16
$H_{end}/\sim H_{end}$	0.76	0.86	0.76	0.11
$E_{end}/\sim E_{end}$	0.78	0.62	0.78	0.11
$C_{end}/\sim C_{end}$	0.72	0.56	0.73	0.16

Table 6.7 Protein ID: AMP-1

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	0.70	0.64	0.70	0.08
$E_{start}/\sim E_{start}$	0.79	0.11	0.82	0.02
$C_{start}/\sim C_{start}$	0.73	0.65	0.73	0.15
$H_{end}/\sim H_{end}$	0.84	0.55	0.85	0.12
$E_{end}/\sim E_{end}$	0.83	0.67	0.83	0.11
$C_{end}/\sim C_{end}$	0.72	0.67	0.72	0.16

Table 6.8 Protein ID: ADD-1

Binary Classifier	Accuracy	Recall	Specifity	Precision
H_{start}/~H_{start}	0.86	0.77	0.87	0.28
E_{start}/~E_{start}	0.84	0.88	0.84	0.11
C_{start}/~C_{start}	0.65	0.66	0.65	0.16
H_{end}/~H_{end}	0.74	0.59	0.75	0.14
E_{end}/~E_{end}	0.86	0.88	0.86	0.13
C_{end}/~C_{end}	0.80	0.37	0.84	0.18

6.10. Test results on randomly chosen subsets of data

In order to test that we did not simply select a subset of the negative data for balancing the dataset that optimized our method's prediction probabilities, we tested our method using 10 different randomly chosen different subsets of the data. These test results show that our proposed method works and that it is possible to train an SVM algorithm to learn where the helices, strands and coils begin and end.

Table 6.9 Test-1, random subset-1

Binary Classifier	Accuracy	Recall	Specifity	Precision
H_{start}/~H_{start}	80	80	80	80
E_{start}/~E_{start}	73	77	68	71
C_{start}/~C_{start}	73	72	73	73
H_{end}/~H_{end}	70	77	64	68
E_{end}/~E_{end}	81	84	78	80
C_{end}/~C_{end}	76	80	72	76

Table 6.10 Test-2, random subset-2

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	81	79	83	83
$E_{start}/\sim E_{start}$	72	78	67	70
$C_{start}/\sim C_{start}$	70	73	68	69
$H_{end}/\sim H_{end}$	73	76	70	72
$E_{end}/\sim E_{end}$	78	82	74	77
$C_{end}/\sim C_{end}$	75	79	71	75

Table 6.11 Test-3, random subset-3

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	87	84	91	90
$E_{start}/\sim E_{start}$	75	74	77	76
$C_{start}/\sim C_{start}$	72	73	71	72
$H_{end}/\sim H_{end}$	74	84	64	70
$E_{end}/\sim E_{end}$	80	83	77	79
$C_{end}/\sim C_{end}$	81	84	77	79

Table 6.12 Test-4, random subset-4

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	84	83	84	84
$E_{start}/\sim E_{start}$	73	71	75	74
$C_{start}/\sim C_{start}$	73	79	68	71
$H_{end}/\sim H_{end}$	76	80	73	75
$E_{end}/\sim E_{end}$	78	84	71	75
$C_{end}/\sim C_{end}$	78	79	77	79

Table 6.13 Test-5, random subset-5

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	80	80	81	81
$E_{start}/\sim E_{start}$	74	74	74	74
$C_{start}/\sim C_{start}$	78	79	77	77
$H_{end}/\sim H_{end}$	76	78	72	81
$E_{end}/\sim E_{end}$	79	82	75	77
$C_{end}/\sim C_{end}$	81	83	79	81

Table 6.14 Test-6, random subset-6

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	82	79	85	84
$E_{start}/\sim E_{start}$	72	70	73	72
$C_{start}/\sim C_{start}$	75	76	74	74
$H_{end}/\sim H_{end}$	79	87	71	75
$E_{end}/\sim E_{end}$	78	81	75	77
$C_{end}/\sim C_{end}$	78	81	74	77

Table 6.15 Test-7, random subset-7

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	83	83	82	81
$E_{start}/\sim E_{start}$	79	86	71	77
$C_{start}/\sim C_{start}$	71	72	70	71
$H_{end}/\sim H_{end}$	73	75	71	72
$E_{end}/\sim E_{end}$	82	82	82	82
$C_{end}/\sim C_{end}$	76	78	73	76

Table 6.16 Test-8, random subset-8

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	84	82	85	85
$E_{start}/\sim E_{start}$	81	84	77	81
$C_{start}/\sim C_{start}$	72	78	66	70
$H_{end}/\sim H_{end}$	74	79	70	72
$E_{end}/\sim E_{end}$	81	85	77	79
$C_{end}/\sim C_{end}$	80	83	76	79

Table 6.17 Test-9, random subset-9

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	85	83	88	87
$E_{start}/\sim E_{start}$	79	84	74	78
$C_{start}/\sim C_{start}$	74	78	69	72
$H_{end}/\sim H_{end}$	74	82	71	66
$E_{end}/\sim E_{end}$	80	84	76	79
$C_{end}/\sim C_{end}$	78	85	71	76

Table 6.18 Test-10, random subset-10

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	83	80	86	85
$E_{start}/\sim E_{start}$	73	73	73	73
$C_{start}/\sim C_{start}$	75	78	73	74
$H_{end}/\sim H_{end}$	73	80	67	70
$E_{end}/\sim E_{end}$	80	84	75	78
$C_{end}/\sim C_{end}$	79	83	75	79

6.11. New binary classifiers tested with the feature selection algorithm

In this section, we apply the feature selection algorithm described in chapter 5 to the boundary detection problem with the new binary classifiers proposed in chapter 6. Table 6.19 shows the Q_T prediction accuracy results of all six binary classifiers--H_{start}/~H_{start}, E_{start}/~E_{start}, C_{start}/~C_{start} and H_{end}/~H_{end}, E_{endt}/~E_{emd} and C_{end}/~C_{end}--used with the SVM RBF kernel and a window size of 9. We used the PSSM profiles of the dataset CB513 during these tests.

As in chapter 5, first, based on the feature set produced by the algorithm, we used this feature set for condensing the PSSM matrix. Our goal was to reduce the feature space of the dataset using the proposed graph-theoretical approach. By merging the vertices within the same clique into one, the original feature space is reduced. Finally, this reduced feature set was applied to a support vector machine algorithm. We were able to achieve similar accuracy results as given in Table 6.1 with less number of features.

Table 6.19 Prediction accuracies of the new binary classifiers with feature selection

Binary Classifier	Accuracy (TP+TN)/ (TP+TN+FN+FP)*	Recall (TP/TP+FN)	Specifity (TN/TN+FP)	Precision (TP/(TP+FP)
H_{start}/~H_{start}	83	81	85	85
E_{start}/~E_{start}	79	84	74	79
C_{start}/~C_{start}	73	74	72	72
H_{end}/~H_{end}	78	84	72	76
E_{end}/~E_{end}	79	85	73	76
C_{end}/~C_{end}	77	79	75	78

6.12. Test results on randomly chosen subsets of data

In order to test that we did not simply select a subset of the data that optimized our method's prediction probabilities when we balanced the dataset, we tested our method using 10 different randomly chosen different subsets of the data. These test results show that our proposed method works and that it is possible to train an SVM algorithm to learn where the helices, strands and coils begin and end when features are reduced based on the clique algorithm.

Table 6.20 Test-1, random subset-1

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	82	80	83	83
$E_{start}/\sim E_{start}$	81	86	74	80
$C_{start}/\sim C_{start}$	73	78	71	68
$H_{end}/\sim H_{end}$	80	85	75	79
$E_{end}/\sim E_{end}$	80	82	77	79
$C_{end}/\sim C_{end}$	77	82	72	76

Table 6.21 Test-2, random subset-2

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	83	80	85	84
$E_{start}/\sim E_{start}$	79	85	72	77
$C_{start}/\sim C_{start}$	73	80	73	70
$H_{end}/\sim H_{end}$	79	86	71	77
$E_{end}/\sim E_{end}$	80	85	74	77
$C_{end}/\sim C_{end}$	81	84	76	80

Table 6.22 Test-3, random subset-3

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	84	82	85	85
$E_{start}/\sim E_{start}$	82	88	75	80
$C_{start}/\sim C_{start}$	76	77	75	75
$H_{end}/\sim H_{end}$	78	86	70	76
$E_{end}/\sim E_{end}$	80	84	75	78
$C_{end}/\sim C_{end}$	77	82	72	76

Table 6.23 Test-4, random subset-4

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	83	80	86	85
$E_{start}/\sim E_{start}$	83	86	79	83
$C_{start}/\sim C_{start}$	76	73	79	78
$H_{end}/\sim H_{end}$	81	88	72	80
$E_{end}/\sim E_{end}$	81	82	80	81
$C_{end}/\sim C_{end}$	79	78	79	80

Table 6.24 Test-5, random subset-5

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	85	82	87	86
$E_{start}/\sim E_{start}$	78	82	74	78
$C_{start}/\sim C_{start}$	72	76	68	71
$H_{end}/\sim H_{end}$	77	81	72	77
$E_{end}/\sim E_{end}$	83	84	81	82
$C_{end}/\sim C_{end}$	80	82	77	79

Table 6.25 Test-6, random subset-6

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	83	80	86	85
$E_{start}/\sim E_{start}$	81	83	78	81
$C_{start}/\sim C_{start}$	74	76	72	73
$H_{end}/\sim H_{end}$	85	89	80	84
$E_{end}/\sim E_{end}$	82	87	78	80
$C_{end}/\sim C_{end}$	78	83	72	77

Table 6.26 Test-7, random subset-7

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	82	79	84	84
$E_{start}/\sim E_{start}$	80	85	74	79
$C_{start}/\sim C_{start}$	71	78	65	69
$H_{end}/\sim H_{end}$	79	85	73	78
$E_{end}/\sim E_{end}$	81	83	79	80
$C_{end}/\sim C_{end}$	77	82	71	76

Table 6.27 Test-8, random subset-8

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	83	83	84	84
$E_{start}/\sim E_{start}$	80	85	75	79
$C_{start}/\sim C_{start}$	77	78	75	76
$H_{end}/\sim H_{end}$	79	85	72	76
$E_{end}/\sim E_{end}$	79	83	75	77
$C_{end}/\sim C_{end}$	80	82	76	80

Table 6.28 Test-9, random subset-9

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	81	81	82	82
$E_{start}/\sim E_{start}$	79	88	70	76
$C_{start}/\sim C_{start}$	74	71	77	76
$H_{end}/\sim H_{end}$	81	88	74	80
$E_{end}/\sim E_{end}$	82	84	81	82
$C_{end}/\sim C_{end}$	80	83	76	79

Table 6.29 Test-10, random subset-10

Binary Classifier	Accuracy	Recall	Specifity	Precision
$H_{start}/\sim H_{start}$	85	83	87	86
$E_{start}/\sim E_{start}$	81	84	77	81
$C_{start}/\sim C_{start}$	74	76	72	73
$H_{end}/\sim H_{end}$	82	85	78	81
$E_{end}/\sim E_{end}$	79	83	76	78
$C_{end}/\sim C_{end}$	79	85	73	77

6.13. Conclusion

In this work, we proposed a new way to look at the protein secondary prediction problem. Most of the current methods use the traditional binary classifiers such as H/~H and require the correct prediction of every residue's secondary structure. This approach gives an overview of the secondary structure of a sequence. However, in order to determine whether a sequence segment is a Helix, Sheet or Coil using the traditional binary classifier, most of the

residues in the sequence segment must be classified correctly. Even with a 90% probability that each residue is correctly predicted independently, the cumulative probability of being correct for all the residues in the sequence segment is low (around 35%). We propose six new binary classifiers that could be used to overcome the problem of classifying all the residues in a given protein sequence segment when we attempt to determine whether the sequence segment is a helix, strand of coil. In our binary classifiers, only one classification is made per segment. In order to use these binary classifiers, we proposed a new encoding scheme for data representation. Our results show that it is possible to train an SVM to learn where the helices, strands and coils begin and end.

CHAPTER 7

Future work

There are many things that can be done to further the research in this dissertation. In chapter 3, we explored the factors involved in the accurate selection of seeds for protein sequence alignments. Our results show that if the proteins in a database are clustered first and a seed search is made, higher quality seeds are found than when an individual database search is made. In the future, PHI-BLAST like algorithms can be improved based on this finding. These algorithms currently do not cluster any of the data and run a search individually for each protein in the whole database. This is not only time consuming, but also it makes it harder for quality seeds to be found.

In chapter 4, we proposed two hybrid kernels SVM_{SM+RBF} and $SVM_{EDIT+RBF}$. Both of these hybrid kernels can be further improved by using different substitution matrices. Also, in both the models, the decision from two kernels are simply added and sent to SVM. However, instead of simply adding these, a different function such a Boolean of two values can be used.

For the feature selection algorithm introduced in chapter 5, the clique-finding approach could be enhanced by using different size cliques for each binary classifier. Our current algorithm uses a fixed size of 5 for all binary classifiers. However, it is possible to achieve higher accuracy if this number is optimized for each classifier individually. Also, different threshold values in the clique-finding algorithm could be applied and tested. Currently we are

using threshold values between -2 and +2 and our algorithm uses a fixed threshold value for all classifiers. Again this value can be adapted to each classifier.

Also, the current feature selection algorithm takes into account only the BLOSUM (BLOcks of Amino Acid SUbstitution Matrix substitution matrix however, many other matrix representations of the protein data could be replaced in our algorithm. Even, these different representations could be combined optimally in the future.

We proposed six new binary classifiers that are used for predicting the starts and end of secondary structures of protein. For these binary classifiers, we also proposed a new encoding scheme. Our current encoding scheme takes only into account the information whether a protein window is the end or start of a secondary structure. It does not take into account where in the protein that sequence window belongs to. Depending on whether it occurs at the beginning or end of a sequence, the occurrence of a transition boundary could be changed drastically. The new binary classifiers currently do not use the information that states a sequence window to be at the start or end of protein; however, they can be improved in the future to represent this information.

Another future improvement could be finding common amino acid patterns that make up the transition boundaries. If there are common amino acid patterns (motifs), this information could be added to the encoding scheme and an SVM could be additionally trained with these patterns to make better prediction. These common patterns could lead to rules as in which order of amino acids represent transition boundaries. These rules can later be embedded into the encoding scheme or put into a new kernel function of SVM.

The correct detection of transition boundaries could be used for predicting the tertiary structure of a protein. For instance, each transition boundary could also be a possible domain

boundary. Proteins are usually made up of several domains or independent functional units which have their own shape and function. All these remain as promising topics for future research.

CHAPTER 8

Conclusion

In this study, first we explored the factors involved in the accurate selection of seeds for protein sequence alignments. We found that it is possible to identify seeds that are likely to share structural similarity with a meaningful *a priori* assessment of accuracy by using a profile-clustered profile approach. In this approach we proposed that instead of searching individual frequency profile of each seed in a database, we should first cluster the database and search for seeds in a clustered database. Based on this finding PHI-BLAST-like algorithms can be substantially improved if the database is clustered first. Our results show that it when sequence windows are clustered and average profiles of these clusters are used for calculating a similarity measure, it is possible to select high quality seeds that share many of the structural properties of a protein.

We also proposed a novel algorithm for feature selection based on cliques and evolutionary information of proteins. We tested our algorithm using random forests, SVM and different encoding schemes for the secondary structure problem in proteins. When we selected only a subset of the features given in the dataset, we found out that the prediction accuracies for both data sets were similar. These results show that our algorithm carefully selects important features whereas unnecessary features were thrown away. Based on the new algorithm we were able to save space and time while still achieving the same accuracy when feature set of the data is not reduced. Using our novel algorithm, we achieved an

approximate 25% reduction in space and time. We tested our algorithm using SVM as a machine learning method instead of random forests and achieved a higher accuracy.

We also propose six new binary classifiers that are used for predicting the starts and end of secondary structures of protein. With these binary classifiers, it is easier to train an SVM since only one prediction per protein segment is necessary for concluding whether it is a helix, strand or coil. In order to use these binary classifiers, we also proposed a new encoding scheme for data representation. Our results show that it is possible to train an SVM to learn where the helices, strands and coils begin and end. We have achieved close to 90% accuracy whereas traditional binary classifiers can only reach to a maximum of 35% accuracy for a window size of 9.

The expected contribution of this dissertation involves two aspects: we develop new methods and algorithms based on statistics, machine-learning and graph-theory approaches for protein structure prediction. In the protein structure prediction problem, we encounter too many negative matches/examples in the data because there are always too many negative samples in the biological dataset compared to positive samples. We tested our methods primarily on protein structure data; however, our methods can be used and tested for different data and applications, such as for gene data.

We also propose methods for predicting protein secondary structure and detecting transition boundaries between the helix, coil and sheet secondary structures. Detecting transition boundaries instead of the structure of individual residues in the whole sequence is much easier. Thus, our problem is reduced to the problem of finding these transition boundaries. Our work provides new insights on accurately predicting protein secondary structure and may help determine the tertiary structure as well; this could be used by

biologists to help solve the critically important problem of how proteins fold. A protein's tertiary structure is critical to its performing its biological functions correctly and efficiently.

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APPENDIX

1. Related papers:

[1] Altun G., Hu H., Gremalschi S., Harrison R.W., Pan Y. A Feature Selection Algorithm based on Graph Theory and Random Forests for Protein Secondary Structure Prediction, *Proc. International Symposium on Bioinformatics Research and Applications (ISBRA'07)*, Lecture Notes in Computer Science, 4463, Springer, pages 590-599, May 2007.

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2. Related posters:

[1] Altun G., Gremalschi S., Harrison R.W., and Zelikovsky A., "Linear Programming for Protein-Protein Interaction Prediction", *2007 Georgia Tech-ONRL International Conference on Bioinformatics,* (BINF 2007).

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