PREDICTION OF ENZYME CLASSES IN A HIERARCHICAL APPROACH BY USING
SPMAP

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ABSTRACT

PREDICTION OF ENZYME CLASSES IN A HIERARCHICAL APPROACH BY USING SPMAP

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Enzymes are proteins that play an important role in biochemical reactions as catalysts. They are classified based on the reaction they catalyzed, in a hierarchical scheme by International Enzyme Commission (EC). This hierarchical scheme is expressed as a four-level tree structure and a unique number is assigned to each enzyme class. There are six major classes at the top level according to the reaction they carried out and sub-classes at the lower levels are further specific reactions of these classes. The aim of this thesis is to build a three-level classification model based on the hierarchical structure of EC classes. ENZYME database is used to extract the information of EC classes and enzymes are assigned to these EC classes. Primary sequences of enzymes extracted from UniProtKB/Swiss-Prot database are used to extract features. A subsequence based feature extraction method, Subsequence Profile Map (SPMap) is used in this study. SPMap is a method that explicitly models the differences between positive and negative examples. SPMap pays attention to the conserved subsequences of protein sequences in the same class. SPMap generates the feature vector of each sample protein as a probability of fixed-length subsequences of this protein with respect to a probabilistic profile matrix calculated by clustering similar subsequences in the training dataset. In our case, positive and negative training datasets are prepared for each class, at each level of

the tree structure. Subsequence Profile Map (SPMap) is used for feature extraction and Support Vector Machines (SVMs) are used for classification. Five-fold cross validation is used to test the performance of the system. The overall sensitivity, specificity and AUC values for the six major EC classes are 93.08%, 98.95% and 0.993, respectively. The results at the second- and third- levels are also promising.

Keywords: enzyme classification, SPMap, subsequence-based feature extraction, Enzyme Commission

ÖZ

SPMAP KULLANARAK ENZİM SINIFLARININ HİYERARŞİK YAKLAŞIMLA TAHMİNİ

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Enzimler biyokimyasal reaksiyonlarda katalizör olarak önemli rol oynayan proteinlerdir. Enzimler, Uluslararası Enzim Komisyonu tarafından, katalize ettikleri reaksiyona bağlı olarak hiyerarşik bir düzende sınıflandırılmışlardır. Bu hiyerarşik şema dört seviyeli bir ağaç yapısı şeklinde ifade edilir ve her bir enzim sınıfı tek bir sayıya atanır. En üst seviyede, yürüttükleri reaksiyona göre başlıca altı sınıf bulunur ve daha aşağı seviyelerdeki alt sınıflar bu sınıfların daha ileri spesifik reaksiyonlarıdır. Bu tezin amacı, Enzim Komisyonu sınıflarının hiyerarşik yapısını kullanarak üç seviyeli bir sınıflandırma modeli inşa etmektir. Enzim Komisyonu sınıf bilgileri ve bu sınıflara ait enzimler ENZYME veritabanı kullanılarak elde edildi. Öznitelikleri oluşturmak için UniProtKB/Swiss-Prot veritabanından çıkarılan enzimlerin birincil dizileri kullanıldı. Bu çalışmada altdizilere dayalı bir öznitelik çıkarma yöntemi olan altdizi profili eşlemesi kullanıldı. Altdizi profili eşlemesi, pozitif ve negatif örnekler arasındaki farkları açıkça modelleyen bir yöntemdir. Bu yöntemde, aynı sınıfa ait protein dizilerinin korunmuş altdizileri dikkate alınır. Her bir protein dizisinin öznitelik vektörü, öğrenme veri kümesindeki benzer altdiziler gruplandırılarak hesaplanan olasılıklı profil matrisine göre proteinin sabit uzunluklu altdizi olasılıkları ile oluşturulur. Bu çalışmada, ağaç yapısındaki her bir seviyedeki her bir sınıf için pozitif ve negatif veri kümeleri hazırlandı. Öznitelikleri çıkarmak için alt-

dizi profili eşlemesi, sınıflandırma için destek vektör makinaları kullanıldı. Sistemin performansını test etmek için beşli çapraz geçerleme kullanıldı. Başlıca altı Enzim Komisyonu sınıfı için elde edilen genel duyarlılık, özgüllük ve ROC eğrisinin altındaki alan değerleri sırayla, 93.08%, 98.95% ve 0.993' tür. İkinci ve üçüncü seviyelerdeki sonuçlar da umut vericidir.

Anahtar Kelimeler: enzim sınıflandırması, SPMMap, altdizi tabanlı öznitelik çıkarımı, Enzim Komisyonu

To my family and to the memory of my father

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Enzymes are proteins that catalyse biochemical reactions by which matter and energy are transformed inside living cells [34]. They lower the activation energy and speed up the rate of the biochemical reactions. Specific reactant molecules called substrates bind to a particular site on the enzyme surface called the active site, where catalytic activities take place. Enzymes bind a substrate to the active site and produce an enzyme-substrate complex, then enzyme-substrate complex breaks to the product and enzyme.

Enzymes don't alter after the reactions and they can then take place in other biochemical reactions.

Enzymes are proteins and proteins are polypeptide chains that are made up of amino acids. There are 20 types of amino acids that naturally occur in the nature (Table 1.1). Amino acids are the basic building blocks of polypeptide chains. There are four levels of protein structures (Figure 1.1). The primary structure of a protein is the chain of amino acids that are linked by peptide bonds. By the development of DNA sequencing methods, three consecutive DNA base sequences are translated to obtain the amino acid sequence of a protein. The amino acid sequence has the information of structural and biological properties and functions of a protein. Secondary structures include α helices, β sheets, loops, turns and other forms that are formed by the interactions between amino acids in the polypeptide. Tertiary structure is the three-dimensional structure of a protein formed by folding of secondary structures of a polypeptide chain. Quaternary structure is the structures of several polypeptide chains inter-

acting with each other. The primary structure of a protein determines the protein's tertiary structure. Polypeptide chains fold into three-dimensional structures and it is this structure that determines the function of an enzyme (i.e. protein).

Table 1.1: List of 20 amino acids.

one-letter abbreviation	three-letter abbreviation	full name
A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

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by Gregory A Petsko and Dagmar Ringe

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Figure 1.1: Levels of protein structures (Adopted from [33]).

Enzymes are classified by International Union of Biochemistry and Molecular Biology (IUBMB) according to the reaction they catalyze. IUBMB developed a hierarchical tree structure (Figure 1.2) with four-levels and a numbering system is used, containing four digits representing each of four levels separated by periods. The type of the reaction is used to assign Enzyme Commission (EC) numbers to enzymes. For an EC number, EC 11.12.13.14; 11 represents one of the six main classes of biochemical reactions, 12 is the subclass by the type of substrate or the bond cleaved, 13 is the sub-subclass by the electron acceptor or the type of group removed and 14 is the sub-sub-subclass that is the serial number of enzyme.

Figure 1.2: Hierarchical tree structure of Enzyme Commission Classes.

The six major Enzyme Commission classes are as follows.

- Oxidoreductases (EC 1) : enzymes catalyzing oxidation reduction reactions.
- Transferases (EC 2) : enzymes catalyzing transfer of functional groups.
- Hydrolases (EC 3) : enzymes catalyzing hydrolysis reactions.
- Lyases (EC 4) : enzymes catalyzing group elimination reactions to form double bonds.
- Isomerases (EC 5) : enzymes catalyzing isomerizations.
- Ligases (EC 6) : enzymes catalyzing bond formation reactions couples with ATP hydrolysis.

1.2 Problem Statement

The number of known enzymes increase rapidly. *In vivo* experiments to decide the function of the newly discovered enzymes are time-consuming and costly. For this reason, computational methods can be used to assign the known enzyme functions to novel enzymes. Computational methods use the structure of the known enzymes, train the classifiers using the features generated from the known enzymes and use the trained classifiers to determine the functions of novel enzymes. Although the function of an enzyme is determined by the tertiary structure, the tertiary structure of most of the enzymes is not known. On the other hand, the primary structure that is the sequence information of most of the enzymes in the ENZYME database can easily be obtained from UniprotKB/Swiss-Prot sequence database. In this study, the primary sequences of enzymes are used to generate features for classification.

Discriminative methods, such as Support Vector Machines (SVMs) construct decision boundaries between positive and negative training samples, therefore selection of positive and negative samples is an important task for these methods to make an accurate distinction between these two set of samples. In our case, for an EC class, C , enzymes belonging to the class C can be used to prepare the positive dataset, but use of all remaining enzymes for negative training dataset would cause an imbalance problem between the datasets. Therefore, representative enzymes should be selected from the remaining EC classes to prepare the negative training dataset.

Another issue for the classification of enzymes is the choice of feature extraction method. In the literature there are a few methods used for functional classification of enzymes using primary sequence information [31]. These are homology-, feature- and subsequence-based approaches. Homology-based approaches use sequence similarity algorithms such as FASTA and BLAST to find homolog proteins of the query protein from a sequence database and annotates the same function to the query protein. This approach cannot be used with discriminative methods since only positive samples are taken into account in the sequence database. Feature-based approaches extract biologically meaningful properties from the primary sequences to represent the proteins. Feature-based approaches use classification algorithms to assign the functions to the proteins. Subsequence-based approaches use conserved subsequences called motifs or domains to represent the protein sequences. Motifs and domains are subsequences among a class of proteins which are known to have important informations about the func-

tions of the proteins. Subsequence-based approaches in the literature use motifs or domains from existing databases which causes computational complexity problems because of the high dimensional and sparse feature vectors.

1.3 Scope

Our aim in this study is to classify enzymes in a top-down manner, using the hierarchical tree structure defined by IUBMB, starting from the first-level and continue with the lower levels. However, the number of enzymes assigned to the classes at the lower levels are not enough to prepare training datasets for classification. Therefore, training is done only with the classes having more than 25 enzymes. It is possible to do classification for the first-, second- and third-level classes with the currently existing number of enzymes at the ENZYME database. There are some classes with less than 25 enzymes at the third-level but this number can still be tolerated. At the fourth-level, the number of enzymes are not enough to perform statistically significant classification.

1.4 Achievements

Enzyme classification studies in the literature perform classification for the first- and second-levels, yet we haven't seen an application of the third-level classification. We performed binary classification for the first-, second- and third-levels of EC hierarchy. The results are promising for all levels. At the first-level, we trained 30 binary classifiers for all 6 EC classes. At the second-level, 285 binary classifiers are trained for 57 sub-classes among 60, which is more than the number of sub-classes employed by previous studies. At the third-level, 700 binary classifiers are trained for 140 sub-subclasses among 207 sub-subclasses which is promising since third-level classification hasn't been investigated before. Totally 1015 binary classifiers are trained for 203 classes in this thesis.

Preparation of positive and negative datasets is an important issue for discriminative models. In our study, we develop an algorithm to prepare representative samples for positive and negative datasets, for each fold of the 5-fold cross-validation, for the EC classes at each level. It is observed that consistent results are produced compared to the classification results of using datasets with randomly selected enzymes. We also use only enzymes to prepare positive

and negative datasets while most of the existing studies use non-enzyme proteins for negative datasets.

In this study, we use a subsequence-based approach called Subsequence Profile Map (SPMap) [35] to extract features for classification which uses information of all fixed-length subsequences of proteins in the training dataset to extract features. In most of the previous approaches, explained in detail in the next chapter, proteins with high sequence identity are not used for training. In our approach, we don't remove these proteins from the training datasets, because SPMap takes into account all the subsequences of the protein sequences in the training dataset and we don't want to disregard any subsequences that may indicate the function of the protein.

The classification system developed in this study can be used by biologists for functional annotation of microbial proteomes most of which are enzymes. The improvements in the sequencing technologies give rise to increment in the number of sequenced microbial genomes [8, 24]. Computational methods are useful to predict the functions of microbial proteomes encoded by these genomes. Automated methods used for genome annotation are reviewed in [41, 30]. Our classification system would be useful for predicting functions of enzymes encoded by newly sequenced microbial organism genomes.

CHAPTER 2

RELATED WORK

2.1 Enzyme Classification in the Literature

In the literature, there are a few studies about the prediction or classification of Enzyme Commission (EC) classes using primary sequence information. We analyzed these studies according to the following criteria :

- employed datasets,
- feature extraction methods (homology-, feature- or subsequence-based approaches),
- classification or prediction systems developed to predict or classify the class of the novel enzyme sequences using the extracted features.

In general, the ENZYME [5] or BRENDA [37] databases were used to extract the information of Enzyme Commission (EC) classes and UniProtKB/Swiss-Prot [44] database was used to extract enzyme sequences.

Chou and Elrod [17] proposed a feature-based prediction system for the second-level sub-classes of oxidoreductases (EC 1), selected from the ENZYME database. The prediction was performed by 16 sub-classes of oxidoreductases, each of which had more than 50 enzymes. The enzyme sequences labeled with these EC sub-classes were extracted from UniProtKB/Swiss-Prot database. Enzymes assigned to multiple classes were removed from the dataset. The feature vectors were generated by using the amino acid composition of the enzyme sequences. The covariant discriminant function algorithm [15] was used to predict the EC class of a query enzyme. The performance was assessed by re-substitution test and jackknife test. The overall

success rate was 75.45% in the re-substitution test and 63.64% in the jackknife test.

The prediction system proposed by Cai et al. [14] used both subsequence- and feature-based approaches. Initially, they performed a prediction between enzymes and non-enzymes and further prediction was made with the six major EC classes. The dataset included 12520 enzyme sequences labeled by the six main EC classes (2314 oxidoreductases, 3653 transferases, 3246 hydrolases, 1307 lyases, 676 isomerases, 1324 ligases) extracted from ENZYME database and 27469 non-enzyme sequences extracted randomly from UniProtKB/Swiss-Prot database. All sequences had less than 60% sequence identity. The enzymes were represented by hybridizing gene product composition and amino acid composition. Gene product composition is a subsequence-based approach, using 1930 dimensional GO_compress database developed by Chou and Cai [18] containing mappings of InterPro functional domain database [2] entries to Gene Ontology (GO) [3]. The enzyme sequences were represented by 1930-dimensional feature vectors, if there was a hit in the InterPro functional domain database. Otherwise, the feature-based approach, amino acid composition [16, 19], was used to represent the enzymes by 57-dimensional vectors. After the generation of feature vectors, Nearest Neighbor (NN) prediction was used to predict the class of a query enzyme. The performance of the system was assessed by re-substitution test, jackknife test and independent test. According to the jackknife test, the success rate for enzymes was 95.61%, non-enzymes was 93.49% and overall success rate was 94.15%. Among the six main enzyme classes, the success rate for oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 4), ligases (EC 5) were 98.98%, 99.13%, 98.87%, 97.18%, 96.77%, 96.75%, respectively and the overall success rate was 98.48%.

Cai and Chou [13] later introduced a subsequence-based approach to make a prediction between enzymes and non-enzymes, and six major Enzyme Commission (EC) classes. 1000 enzymes, having less than 20% sequence identity were extracted from the ENZYME database and 1301 non-enzyme protein sequences with less than 20% sequence identity were extracted from UniProtKB/Swiss-Prot database. 7785 functional domains extracted from the InterPro functional domain database were used to represent the proteins as 7785-dimensional feature vectors. The EC classes of a novel protein was predicted by Nearest Neighbor (NN) method. The overall success rate was 85.35%.

Chou [19] introduced the amphiphilic pseudo amino acid composition (Am-Pse-AA) to represent enzyme sequences. Am-Pse-AA had information about the order of protein sequence and distribution of the hydrophobic and hydrophilic amino acids along the chain. The same dataset (16 sub-classes of oxidoreductases) constructed by Chou and Elrod [17] was used to compare the performance of the method with the previous one. The enzyme classes were predicted by the covariant discriminant algorithm. The overall success rate of the re-substitution test was 92.16% and the jackknife test was 70.61%.

EzyPred is a 3-layer online prediction tool developed by Shen and Chou [40] that makes a prediction between enzymes and non-enzymes at the 1st layer, six major enzyme classes at the 2nd layer and sub-classes of the major enzyme classes at the 3rd layer. To prepare the datasets, they extracted 9850 non-enzyme protein sequences randomly from the UniProtKB/Swiss-Prot database. The enzymes for enzyme classes were extracted from ENZYME database and enzyme sequences with less than 50 amino acids, having multiple enzyme classes or having more than 40% sequence identity were removed. 9832 enzymes labeled with six main enzyme classes were obtained. The sub-classes having more than 10 enzyme sequences were used for statistical significance. Two feature vectors were generated for protein sequences, using the Functional Domain (FunD) approach and Pseudo Position-Specific Scoring Matrix (Pse-PSSM) approach. In the FunD approach, 8958 functional domains were extracted from Pfam database [6]. A protein sequence was represented by an 8958-dimensional feature vector, each entry taking the value 1 if the corresponding domain exists in the protein sequence, otherwise 0. Another representation of the protein sequence was by pseudo position-specific scoring matrix (Pse-PSMM) adopted from [16] and [19]. A modified version of k Nearest Neighbor classifier, called optimized evidence-theoretic k nearest neighbor (OET-KNN) classifier [21, 38, 45] was used for the prediction of a novel protein sequence. The OET-KNN classifier was trained by the FunD feature vector and Pse-PSSM feature vector, a voting score was calculated by fusing the results and the class of the query protein was predicted as the one with the highest score. Figure 2.1 illustrates the fusion approach and the 3-layer prediction approach. The success rate obtained by the jackknife test was 92.4% for enzymes and 90.1% for non-enzymes. The success rate of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6) were 91.4%, 94.5%, 97.1%, 85.1%, 83.6% and 96.5%, respectively. The overall success rate was 93.7%. The overall success rates of sub-classes of oxidoreductases, transferases, hydrolases, lyases, isomerases

and ligases were 86.7%, 95.8%, 95.9%, 94.4%, 93.3% and 98.3%, respectively.

Figure 2.1: A flowchart to show (A) how to use the FunD approach and Pse-PSSP approach into a prediction engine, and (B) how the top-down approach of the 3-layer predictor works (Adopted from [40])

Cai et al. [11, 12] developed a web-based discriminative classification system, SVM-Prot, for the functional classification of proteins from primary sequences. They prepared positive and negative datasets for different functional families of enzymes beyond Enzyme Commission classes. Enzyme families (i.e. Enzyme Commission classes) included 46 second-level sub-classes extracted from BRENDA database [37]. The positive training dataset contained the proteins assigned to an enzyme family and the negative training samples for this family were extracted from protein families in the Pfam database [6] including both enzymes from other enzyme families and non-enzyme proteins. The protein features were represented by the combination of composition, transition and distribution descriptors that described the composition of physicochemical properties of residues in the protein sequences such as amino acid composition, hydrophobicity, normalized Van der Waals volume, polarity, polarizability, charge, surface tension, secondary structure and solvent accessibility. The classifier was trained with positive and negative datasets by Support Vector Machines (SVMs) to classify novel protein sequences. The sensitivity, specificity and Matthews correlation coefficient values for the 46 Enzyme Commission (EC) sub-classes were in the range of 50% to 95.7%, 79.0% to 96.1%, and 54.1% to 96.1%, respectively.

Lu et al. [29] developed an online Enzyme Classification System (ECS) to identify enzymes and non-enzymes as well as six enzyme classes using a subsequence-based approach based on

the functional domain composition of proteins. ENZYME database was used to prepare the positive dataset (enzymes) and UniProtKB/Swiss-Prot database was used to prepare the negative dataset (non-enzymes). 70573 enzymes were extracted for six major enzyme classes and 145271 proteins were extracted for non-enzymes. 2657 functional domains were extracted from Pfam database for enzymes and non-enzymes and each protein was represented with a 2657-dimensional feature vector. Support Vector Machines (SVMs) were used for classification. According to jackknife test the success rate of enzymes was 76.30%, non-enzymes was 90.89% and success rate of the overall prediction was 86.03%. Success rate for oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6) were 93.35%, 93.63%, 94.20%, 75.29%, 74.56% and 89.33%, respectively. The overall success rate of six main enzyme classes was 91.32%.

One of the earliest studies about prediction of enzyme classes was a homology based approach presented by Shah and Hunter [39]. Sequence similarity methods were applied to predict Enzyme Commission (EC) classes. The number of enzymes assigned to the EC classes and the number of subclasses were very fewer compared to the numbers we used in this thesis. The number of enzymes labeled with oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases were 3766, 4363, 4649, 1604, 576 and 655, respectively, whereas in our study these numbers are increased to 28539, 49425, 29890, 14264, 8225 and 18336, respectively. They applied the sequence database similarity search algorithms FASTA [32] and BLAST [1] to predict the EC classes of 13215 enzyme sequences extracted from UniProtKB/Swiss-Prot labeled with EC numbers. Because of the under sampling problem they couldn't cover all enzyme classes. The performance of the system was assessed by FASTA z-score and BLAST expectation measures. There wasn't a significant difference between the performances of FASTA and BLAST. Over 13215 proteins, the EC classes of 12150 proteins were correctly predicted.

Table 2.1 summarizes the key aspects of previous studies according to the datasets, feature extraction methods and prediction or classification methods.

Table 2.1: Review of Studies Involving Prediction of Enzyme Classes. (The abbreviations are, GO : Gene Ontology, PseAA : Pseudo Amino Acid composition, FunD : Functional Domain, Am-Pse-AA : Amphiphilic Pseudo Amino Acid composition, Pse-PSSM : Pseudo Position-Specific Scoring Matrix, CDA : Covariant Discriminant Algorithm, NN : Nearest Neighbor, OET-KNN : Optimized Evidence-Theoretic k Nearest Neighbor, SVMs : Support Vector Machines).

Name	Dataset	Feature Extraction	Prediction or Classification
Chou and Elrod (2003) [17]	16 sub-classes of EC 1	amino acid composition	CDA
Cai et al. (2004) [14]	non-enzymes 6 major EC classes	GO-PseAA	NN
Cai and Chou (2005) [13]	non-enzymes 6 main EC classes	FunD InterPro database	NN
Chou (2005) [19]	16 sub-classes of EC 1	Am-Pse-AA	CDA
Shen and Chou (2007) [40]	non-enzymes 6 major EC classes second-level sub-classes	FunD Pse-PSSM	OET-KNN
Lu et al. (2007) [29]	non-enzymes 6 major EC classes	FunD Pfam	SVMs
Shah and Hunter (1997) [39]	all EC classes	sequence similarity	FASTA and BLAST

2.2 Differences of our study from existing methods

- We train binary classifiers for the third-level EC sub-subclasses beyond the first-level EC classes and second-level EC subclasses.
- Subsequence-based approaches in the literature use subsequences already available at the motif or domain databases. In our study, subsequences extracted from the training dataset are used to generate the feature vectors.
- An algorithm is developed to prepare positive and negative training datasets for the binary classifiers, in order to extract representative enzymes from all sub-sub-subclasses on the hierarchical tree structure to improve the predictive performance of the classification system.

- Most of the existing approaches remove the proteins with high sequence identity from the training datasets. We didn't make such a restriction while preparing the training datasets not to lose the subsequences that might be important while extracting features by SPMMap.
- Preparation of negative datasets is not explained clearly, in the previous studies.

CHAPTER 3

DATASETS AND METHODS

3.1 DATASETS

In this study, a three-level binary classification system is developed using the hierarchical tree structure of the Enzyme Commission (EC) classes defined by Enzyme Nomenclature Committee of International Union of Biochemistry and Molecular Biology (IUBMB) [23]. There are four levels in the tree structure of the hierarchical scheme. Positive and negative datasets are prepared for 6 classes at the first-level, 57 sub-classes at the second-level and 140 sub-subclasses at the third-level. The sub-sub-subclasses at the fourth-level don't have enough number of samples, therefore they are not considered. Subsequence Profile Map (SPMAP) [35] is used for feature extraction and Support Vector Machines (SVM) [22] are used for classification. Five-fold cross-validation is used to test the performance of the system.

EC numbers and Uniprot/Swiss-Prot accession numbers of enzymes are extracted from the ENZYME database (release of 20-Jan-2009) [5]. Table 3.1 shows a sample ENZYME database entry. Only, ID and DR lines are used to extract information for the preparation of datasets. The ID (IDentification) line gives the EC number. The EC numbers at the ENZYME database are identified as EC a.b.c.d. Here 'a' is the number of the class at the first-level, 'b' is the number of the sub-class at the second-level, 'c' is the number of the sub-subclass at the third-level and 'd' is the number of the sub-sub-subclass at the fourth-level. The DR (Database Reference) lines contain the UniProtKB/Swiss-Prot accession numbers of enzymes assigned to the EC numbers. The primary sequences of enzymes are extracted from UniProtKB/Swiss-Prot (release 14.7 20-Jan2009).

Table 3.1: An example ENZYME database entry.

```
//
ID    1.1.1.2
DE    Alcohol dehydrogenase (NADP(+)).
AN    Aldehyde reductase (NADPH).
CA    An alcohol + NADP(+) = an aldehyde + NADPH.
CF    Zinc.
CC    -!- Some members of this group oxidize only primary alcohols; others act
CC    also on secondary alcohols.
CC    -!- May be identical with EC 1.1.1.19, EC 1.1.1.33 and EC 1.1.1.55.
CC    -!- A-specific with respect to NADPH.
PR    PROSITE; PDOC00061;
DR    P35630, ADH1_ENTHI ; Q24857, ADH3_ENTHI ; Q04894, ADH6_YEAST ;
DR    P25377, ADH7_YEAST ; O57380, ADH8_RANPE ; P0A4X1, ADHC_MYCBO ;
DR    P0A4X0, ADHC_MYCTU ; P25984, ADH_CLOBE ; P75214, ADH_MYCPN ;
DR    P14941, ADH_THEBR ; Q3ZCJ2, AK1A1_BOVIN; O70473, AK1A1_CRIGR;
DR    P14550, AK1A1_HUMAN; Q9JII6, AK1A1_MOUSE; P50578, AK1A1_PIG ;
DR    P51635, AK1A1_RAT ; Q9UUN9, ALD2_SPOSA ; P27800, ALDX_SPOSA ;
//
```

Table 3.2 shows the total number of enzymes assigned to six major enzyme classes and number of sub-classes of these classes at the lower levels. For example, oxidoreductases have 21 sub-classes at the second-level, 85 sub-subclasses at the third level, 617 sub-sub-subclasses at the fourth-level and in total 28539 enzymes assigned to the sub-sub-subclasses.

Table 3.2: First-level EC classes and their related information.

EC class	number of sub-classes at level			total number of proteins
	2	3	4	
1 Oxidoreductases	21	85	617	28539
2 Transferases	9	31	686	49425
3 Hydrolases	11	49	730	29890
4 Lyases	7	15	221	14264
5 Isomerases	6	17	103	8225
6 Ligases	6	10	97	18336
Total	60	207	2454	148679

3.1.1 Data Preparation for the First-Level Classification

There are 6 major EC classes at the first level of the hierarchy. To prepare positive and negative training datasets for each of these 6 classes, positive samples are extracted from the target EC class and negative samples are extracted from the remaining five EC classes.

Figure 3.1 is a sample representation of the tree structure showing the positive and negative EC classes selected to prepare the training datasets for a first-level target EC class, EC 1 (oxidoreductases).

Positive datasets for each fold of the 5-fold cross-validation are prepared by selecting enzymes from the sub-sub-subclasses of EC 1 and negative datasets are prepared by selecting representative enzymes from the sub-sub-subclasses of remaining 5 major EC classes.

Algorithm 1 is used to prepare positive datasets for each fold of the 5-fold cross-validation. The classification performance would be comparable if every fold contains enzymes from each sub-sub-subclass of the target EC class. Therefore, for each target EC class p , for each sub-sub-subclass having $size$ enzymes, if $size$ is more than five, $size/5$ of the enzymes are added to each fold. If $size$ is less than five, these enzymes are added to the temporary set *tempprots*. After selecting enzymes from all of the sub-sub-subclasses, $1/5$ of the enzymes in *tempprots* are added to each fold.

Figure 3.1: Graphical representation of the tree structure showing the positive and negative EC sub-sub-classes selected to prepare training datasets for EC 1 - Oxidoreductases (The nodes with blue, solid lines represent the positive classes and the nodes with red, dashed lines represent the negative classes).

Algorithm 1 Pseudo code for the algorithm to prepare positive datasets for the first-level EC classes.

```
 $C \leftarrow$  set of the first-level EC classes  
 $p \leftarrow$  target EC class  
 $p \in C$   
 $tempprots$  is the temporary set of enzymes  
 $tempprots \leftarrow \emptyset$   
for all forth-level sub-sub-subclasses of  $p$  do  
   $size \leftarrow$  number of enzymes assigned to this class  
  if  $size > 5$  then  
    add ( $size/5$ ) of the enzymes to each fold  
  else  
    add enzymes to  $tempprots$   
  end if  
end for  
add  $1/5$  of  $tempprots$  to each fold
```

Algorithm 2 is used to prepare negative datasets for each fold of the 5-fold cross-validation. For each target EC class p , enzymes from the remaining five EC classes N are selected for the negative datasets. However, if all the enzymes from the negative classes were selected, the total number of enzymes in the negative datasets would be much more than the enzymes in the positive datasets, which will cause an imbalance between the datasets. Therefore, for the sub-sub-subclasses having more than 25 enzymes, $1/3$ of the enzymes are selected and $1/5$ of these enzymes are added to each fold. Otherwise, if the size of the enzymes is more than five, $size/5$ of the proteins are added to each fold, if $size$ is less than five, the enzymes are added to $tempprots$. After selecting all enzymes, $1/5$ of the enzymes in $tempprots$ are added to each fold. By doing this, the negative training dataset consists representative samples from all of the sub-sub-subclasses at the fourth-level.

3.1.2 Data Preparation for the Second-Level Classification

Table 3.3 shows the second-level EC sub-classes and number of proteins in each second-level sub-class.

Table 3.3: Second-Level EC sub-classes and number of proteins.

EC 1 Oxidoreductases		
sub-class	Name	number of proteins
EC 1.1	Acting on the CH-OH group	6047
EC 1.2	Acting on the aldehyde or oxo group of donors	2483
EC 1.3	Acting on the CH-CH group of donors	1589
EC 1.4	Acting on the CH-NH ₂ group of donors	1121
EC 1.5	Acting on the CH-NH group of donors	6618
EC 1.6	Acting on NADH or NADPH	3248
EC 1.7	Acting on other nitrogenous compounds as donors	718
EC 1.8	Acting on a sulfur group of donors	953
EC 1.9	Acting on a heme group of donors	588
EC 1.10	Acting on diphenols and related substances as donors	190
EC 1.11	Acting on a peroxide as acceptor	1370
EC 1.12	Acting on hydrogen as donor	83
EC 1.13	Acting on single donors with incorporation of molecular oxygen (oxygenases)	609
EC 1.14	Acting on paired donors, with incorporation or reduction of molecular oxygen	1214
EC 1.15	Acting on superoxide radicals as acceptor	426
EC 1.16	Oxidising metal ions	110
EC 1.17	Acting on CH or CH ₂ groups	814
EC 1.18	Acting on iron-sulfur proteins as donors	208
EC 1.20	Acting on phosphorus or arsenic in donors	16
EC 1.21	Acting on X-H and Y-H to form an X-Y bond	71
EC 1.97	Other oxidoreductases	63

Table 3.3 - Continued

EC 2 Transferases		
sub-class	Name	number of proteins
EC 2.1	Transferring one-carbon groups	6740
EC 2.2	Transferring aldehyde or ketonic groups	1032
EC 2.3	Acyltransferases	4457
EC 2.4	Glycosyltransferases	5817
EC 2.5	Transferring alkyl or aryl groups, other than methyl groups	4156
EC 2.6	Transferring nitrogenous groups	1582
EC 2.7	Transferring phosphorus-containing groups	24629
EC 2.8	Transferring sulfur-containing groups	930
EC 2.9	Transferring selenium-containing groups	82
EC 3 Hydrolases		
sub-class	Name	number of proteins
EC 3.1	Acting on ester bonds	9480
EC 3.2	Glycosylases	2551
EC 3.3	Acting on ether bonds	255
EC 3.4	Acting on peptide bonds (peptidases)	4999
EC 3.5	Acting on carbon-nitrogen bonds, other than peptide bonds	5720
EC 3.6	Acting on acid anhydrides	6708
EC 3.7	Acting on carbon-carbon bonds	89
EC 3.8	Acting on halide bonds	41
EC 3.10	Acting on sulfur-nitrogen bonds	1
EC 3.11	Acting on carbon-phosphorus bonds	43
EC 3.13	Acting on carbon-sulfur bonds	3

Table 3.3 - Continued

EC 4 Lyases		
sub-class	Name	number of proteins
EC 4.1	Carbon-carbon lyases	5082
EC 4.2	Carbon-oxygen lyases	6881
EC 4.3	Carbon-nitrogen lyases	944
EC 4.4	Carbon-sulfur lyases	386
EC 4.5	Carbon-halide lyases	2
EC 4.6	Phosphorus-oxygen lyases	520
EC 4.99	Other lyases	449
EC 5 Isomerases		
sub-class	Name	number of proteins
EC 5.1	Racemases and epimerases	1381
EC 5.2	cis-trans-Isomerases	689
EC 5.3	Intramolecular isomerases	3307
EC 5.4	Intramolecular transferases(mutases)	2358
EC 5.5	Intramolecular lyases	104
EC 5.99	Other isomerases	386
EC 6 Ligases		
sub-class	Name	number of proteins
EC 6.1	Forming carbon-oxygen bonds	9505
EC 6.2	Forming carbon-sulfur bonds	808
EC 6.3	Forming carbon-nitrogen bonds	6585
EC 6.4	Forming carbon-carbon bonds	649
EC 6.5	Forming phosphoric ester bonds	747
EC 6.6	Forming nitrogen-metal bonds	42

In the second-level, there are 60 sub-classes. EC 3.10, EC 3.13 and EC 4.5 sub-classes don't have sufficient number of enzymes, therefore positive and negative datasets are prepared for 57 sub-classes.

The algorithms used to prepare positive and negative datasets for the second-level sub-classes

are similar to the ones explained at the first-level data preparation. The difference from the first-level is that, in the first-level classification, 1/3 of the enzymes are selected from the negative classes, but in the second-level classification all of the enzymes in the negative sub-classes are used to prepare the negative datasets. At the second-level, one second-level sub-class of a first-level class is used as the target sub-class, and the remaining second-level sub-classes of the same class are used to prepare the negative datasets for the target sub-class. For example, Oxidoreductases has 21 sub-classes at the second-level. For the target class, EC 1.1, the positive datasets are prepared using this sub-class and the negative datasets are prepared using the remaining 20 sub-classes of oxidoreductases. Figure 3.2 shows the positive and negative EC sub-sub-subclasses selected to prepare training datasets for EC 1.1 (Oxidoreductases acting on the CH-OH group), on the tree structure. Algorithm 3 is the pseudo code for the algorithm to prepare positive datasets for the second-level EC sub-classes and Algorithm 4 is the pseudo code for the algorithm to prepare negative datasets for the second-level EC sub-classes.

Figure 3.2: Graphical representation of the tree structure showing the positive and negative EC sub-sub-subclasses selected to prepare training datasets for EC 1.1 - Oxidoreductases acting on the CH-OH group (The nodes with blue, solid lines represent the positive classes and the nodes with red, dashed lines represent the negative classes).

Algorithm 2 Pseudo code for the algorithm to prepare negative datasets for the first-level EC classes.

$C \leftarrow$ set of the first-level EC classes
 $p \leftarrow$ target EC class
 $N \leftarrow$ set of the negative EC classes
 $N \leftarrow C - p$
 $n \leftarrow$ negative EC class
 $n \in N$
 $tempprots$ is the temporary set of enzymes
 $tempprots \leftarrow \emptyset$
for all first-level EC class n **do**
 for all fourth-level sub-sub-subclasses of n **do**
 $size \leftarrow$ number of enzymes assigned to this class
 if $size < 25$ **then**
 if $size > 5$ **then**
 add $(size/5)$ of the enzymes to each fold
 else
 add enzymes to $tempprots$
 end if
 else
 select random $(size/3)$ enzymes
 $size \leftarrow size/3$
 add $(size/5)$ of the enzymes to each fold
 end if
 end for
end for
add 1/5 of $tempprots$ to each fold

Algorithm 3 Pseudo code for the algorithm to prepare positive datasets for the second-level EC sub-classes.

$E \leftarrow$ first-level enzyme class
 $p \leftarrow$ second-level target sub-class of E
 $tempprots$ is the temporary set of enzymes
 $tempprots \leftarrow \emptyset$
for all forth-level sub-sub-subclasses of p **do**
 $size \leftarrow$ number of proteins assigned to this class
 if $size > 5$ **then**
 add (size/5) of the proteins to each fold
 else
 add proteins to $tempprots$
 end if
end for
add 1/5 of $tempprots$ to each fold

Algorithm 4 Pseudo code for the algorithm to prepare negative datasets for the second-level EC sub-classes.

$E \leftarrow$ first-level enzyme class

$p \leftarrow$ second-level target sub-class of E

$N \leftarrow$ set of negative sub-classes

$n \leftarrow$ negative sub-class

$n \in N$

$tempprots$ is the temporary set of proteins

$tempprots \leftarrow \emptyset$

for all second-level EC sub-class n **do**

for all fourth-level sub-sub-subclasses of n **do**

$size \leftarrow$ number of proteins assigned to this class

if $size > 5$ **then**

 add $(size/5)$ of the proteins to each fold

else

 add proteins to $tempprots$

end if

end for

end for

add $1/5$ of $tempprots$ to each fold

3.1.3 Data Preparation for the Third-Level Classification

There are 207 sub-sub-subclasses at the third-level of the hierarchical tree structure of the Enzyme Commission classes. The sub-classes EC 1.9, EC 1.11, EC 1.15, EC 1.97, EC 2.2, EC 2.5, EC 2.9, EC 3.7, EC 3.8, EC 3.11, EC 4.4, EC 4.6, EC 4.99, EC 5.2, EC 5.5, EC 5.99, EC 6.1, EC 6.2, EC 6.4, EC 6.5 and EC 6.6 have only one sub-subclass at the third level, therefore it is redundant to perform classification for the sub-subclasses of these sub-classes at the third-level again. Positive and negative datasets are prepared for 140 sub-subclasses from the remaining 186 classes, which have sufficient number of enzymes to train the classifiers. The same algorithm explained for the second-level data preparation is applied to prepare the datasets at the third-level. Figure 3.3 shows the positive and negative EC sub-sub-subclasses selected to prepare training datasets for an example sub-subclass, EC 1.1.1 (Oxidoreductases acting on the CH-OH group with NAD or NADP as acceptor), on the tree structure.

Figure 3.3: Graphical representation of the tree structure showing the positive and negative EC sub-sub-subclasses selected to prepare training datasets for EC 1.1.1 - Oxidoreductases acting on the CH-OH group with NAD or NADP as acceptor (The nodes with blue, solid lines represent the positive classes and the nodes with red, dashed lines represent the negative classes).

3.2 METHODS

Subsequence Profile Map (SPMap) [35] is used for feature extraction and Support Vector Machines (SVM) [22] are used for classification.

3.2.1 SPMap

SPMap is a subsequence-based feature extraction method which uses all fixed-length subsequences in the training dataset to construct a probabilistic profile map and calculates the feature values forms feature vector of each protein according to these profiles. There are 4 steps for feature extraction in SPMap.

- Subsequence Extraction : In the first step, all fixed length subsequences of proteins in the training dataset are extracted.
- Clustering : In the second step, similar subsequences are clustered.
- Probabilistic Profile Matrix Construction : In the third step, a probabilistic profile matrix is constructed for each cluster.
- Feature Vector Generation : In the fourth step, feature vectors of each protein in the training dataset are constructed.

Figure 3.4 shows these steps.

Subsequence Extraction

Conserved subsequences of proteins called motifs are known to have important role on identifying the functions of proteins, because rather than other parts of sequences, regions such as catalytic sites and binding sites are conserved over much wider taxonomic distances [7]. There are several motif and domain databases such as PROSITE [27], BLOCKS [26], PRINTS [4], InterPro [2], Pfam [6] and ProDom [20] that are used to construct feature vectors. In SPMap, instead of using motifs or domains from these databases, fixed length subsequences are extracted from the training datasets of protein sequences and information coming from these subsequences is used to generate the feature vectors.

Figure 3.4: Steps and general flow of SPMAP. 1) Subsequence extraction. 2) Clustering. 3) Probabilistic profile matrix construction. 4) Feature vector generation.

Clustering

Clustering is the crucial part of SPMaP method. The flowchart of the clustering algorithm used in SPMaP is shown in Figure 3.5.

Figure 3.5: Flowchart of the clustering algorithm.

After the extraction of 5-length subsequences from the training dataset, these subsequences are clustered according to the similarity among them. Initially, the first subsequence, ss_1 , is added to the first cluster, c_1 . Then each subsequence, ss_i , is compared with the subsequences in all clusters, $c_k, k : 1, \dots, n$. The average similarity, avg_k , between the subsequence, ss_i and the subsequences in cluster, c_k , is calculated by the formula,

$$avg_k = \frac{\sum_{ss_j \in c_k} s(ss_i, ss_j)}{|c_k|} \quad (3.1)$$

where $s(ss_i, ss_j)$ is the similarity between two subsequences, ss_i and ss_j . The similarity matrix, Blosum62, shown in Figure 3.6 is used while calculating the similarity between two subsequences. For the 5-length two subsequences, x and y , the similarity between them is the sum of the similarity values of amino acids at each position of the subsequences, in Blosum62 matrix as given in Equation 3.2.

$$s(x, y) = \sum_{i=1}^5 M(x(i), y(i)) \quad (3.2)$$

For example, for two subsequences,

$ss_1 = "MTKGP"$ and $ss_2 = "MKNKS"$, the similarity between them is

$$s(ss_1, ss_2) = M(M, M) + M(T, K) + M(K, N) + M(G, K) + M(I, S)$$

$$= 5 + (-1) + 0 + (-2) + (-2)$$

$$= 0$$

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Figure 3.6: Blosom62 amino acid similarity matrix [25].

After calculating the average similarity values for all clusters, the subsequence is added to the cluster, c_m , with the maximum average similarity value, avg_m , if this value is greater than the threshold value, δ . Otherwise, a new cluster, c_{n+1} , is created and the subsequence, ss_i , is added to this cluster. The threshold value is important because the number of clusters, n , depends on the threshold value, δ . A small threshold value will cause dissimilar subsequences fall into the same cluster. On the other hand, the number of clusters, hence the dimension of the feature space will increase with a high threshold value. In this study, the threshold value δ is chosen as 8.

Probabilistic Profile Matrix Construction

The third step of the SPMMap method is the generation of probabilistic profiles from the clusters created in the second step. For each cluster, c_k , a probabilistic profile, PP_k -a matrix with 5x20 dimensions- is generated, where 5 is the length of the subsequences and 20 is the number of amino acids. Each (i, j) th entry of the probabilistic profile, PP_k , is calculated by Equation 3.3 where $\phi_k(i, j)$ is the count of the j th amino acid at i th position of all of the subsequences in cluster, c_k . κ is used to overcome overfitting and zero probability problems.

$$PP_k(i, j) = \log \frac{\phi_k(i, j) + \kappa}{|c_k|} \quad (3.3)$$

Feature Vector Generation

The feature vector of an enzyme sequence is generated by distributing its subsequences over the subsequence profile map constructed in the clustering step. First, all 5-length subsequences of the enzyme sequence are extracted. Each of these subsequences are compared with the probabilistic profile, PP_k , created for each cluster, c_k and a probability is calculated based on Equation 3.4.

$$P(ss|PP_k) = \sum_{i=0}^5 PP_k(i, ss(i)) \quad (3.4)$$

Here, the probability of the subsequence ss to appear in cluster c_k is the sum of the probabilistic profile values for i th amino acid of ss at the i th position of all subsequences in cluster c_k .

The dimension of the feature vector, V , of an enzyme sequence is equal to the number of clusters. Each k th value of V is the probability of the subsequence of enzyme S with the highest score on the probabilistic profile PP_k defined by:

$$V(k) = \max_{ss_i \in S} P(ss_i|PP_k) \quad (3.5)$$

If the probability is very small, k th value of the feature vector $V(k)$ is set to zero.

3.2.2 Classification

After generating the feature vectors from enzyme sequences in the training dataset, Support Vector Machines (SVMs) are applied to the enzyme classification problem. Radial Basis Function (RBF) is used as the kernel function for SVM. The SVM parameter C and RBF kernel parameter γ are set to 2 and 0.005, respectively. SVM-light software by Joachims (1999) [28] is used to implement SVM.

Support Vector Machines are binary classifiers introduced by Vapnik [42, 43, 36].

The notation is adopted from Burges [10].

Let l be the number of samples in the training dataset and $\mathbf{x}$ be the feature vectors representing these samples (in our case enzymes). The training data is labeled as follows:

$$\{\mathbf{x}_i, y_i\}, \quad i = 1, \dots, l, \quad y_i \in \{-1, +1\}, \quad \mathbf{x}_i \in \mathbb{R}^d$$

where $\mathbf{x}_i$ is the vector representing the i th sample and y_i is the label of the sample indicating the class it belongs to (+1 for positive and -1 for negative).

3.2.2.1 Linear Support Vector Machines

Linear Separable SVM

In the linear separable case (Figure 3.7), the goal of SVM is to find a hyperplane

$$\mathbf{w} \cdot \mathbf{x} + b = 0 \tag{3.6}$$

that separates the positive and negative samples, where

$\mathbf{w}$: normal to the hyperplane,

$\frac{|b|}{\|\mathbf{w}\|}$: the perpendicular distance from the hyperplane to the origin,

$\|\mathbf{w}\|$: Euclidean norm of $\mathbf{w}$.

The decision function is $f(\mathbf{x}) = \text{sign}(\mathbf{w} \cdot \mathbf{x} + b)$. If $f(\mathbf{x}) > 0$, $\mathbf{x}$ is classified to the positive class ($y = +1$), otherwise the negative class ($y = -1$).

Figure 3.7: Linear separating hyperplanes for separable SVM. (Adopted from [10])

Let

d_+ : the shortest distance from the separating hyperplane to the closest positive example,

d_- : the shortest distance from the separating hyperplane to the closest negative example,

$d_+ + d_-$: the "margin" of the separating hyperplane.

The optimal hyperplane is the hyperplane that separates the positive samples from the negative samples with maximal margin. In order to maximize the margin, the separating hyperplane should satisfy the constraints

$$\mathbf{x}_i \cdot \mathbf{w} + b \geq +1 \quad \text{for } y_i = +1 \quad (3.7)$$

$$\mathbf{x}_i \cdot \mathbf{w} + b \leq -1 \quad \text{for } y_i = -1 \quad (3.8)$$

which can be combined into the inequality

$$y_i(\mathbf{x}_i \cdot \mathbf{w} + b) - 1 \leq 0 \quad \forall i \quad (3.9)$$

The two hyperplanes H_1 and H_2 , where the closest positive and negative data points lie on are

$$H_1 = \mathbf{x}_i \cdot \mathbf{w} + b = 1 \quad (3.10)$$

and

$$H_2 = \mathbf{x}_i \cdot \mathbf{w} + b = -1 \quad (3.11)$$

where $\mathbf{w}$ is the normal, $|1 - b|/\|\mathbf{w}\|$ is the perpendicular distance of H_1 from the origin, $|-1 - b|/\|\mathbf{w}\|$ is the perpendicular distance of H_2 from the origin and $2/\|\mathbf{w}\|$ is the margin of the

separating hyperplane (the distance between H_1 and H_2). In order to maximize the margin the optimization problem

$$\min_{\mathbf{w}, b} \frac{1}{2} \|\mathbf{w}\|^2 \quad (3.12)$$

should be solved, subject to the constraints in Equation 3.9. The points that lie on H_1 , H_2 (indicated by circles in Figure 3.7) and satisfy these constraints are called *support vectors*.

Now, the problem of constructing an optimal separating hyperplane turns into a convex quadratic problem. Lagrange multipliers $\alpha_i > 0$ are introduced to solve this problem. The Lagrangian formulation of the minimization problem is

$$\min_{\mathbf{w}, b, \alpha} L_P \equiv \frac{1}{2} \|\mathbf{w}\|^2 - \sum_{i=1}^l \alpha_i (y_i (\mathbf{x}_i \cdot \mathbf{w} + b) - 1) \quad (3.13)$$

with respect to $\mathbf{w}$ and b , subject to constraints

$$\mathbf{w} = \sum_{i=1}^l \alpha_i y_i \mathbf{x}_i \quad (3.14)$$

$$\sum_{i=1}^l \alpha_i y_i = 0 \quad (3.15)$$

where $\alpha_i, i = 1, \dots, l$ are the Lagrange multipliers for inequality constraints in Equation 3.9.

The problem is transformed into its dual optimization problem of maximizing L_D ,

$$\max_{\alpha} L_D = \sum_{i=1}^l \alpha_i - \frac{1}{2} \sum_{i,j=1}^l \alpha_i \alpha_j y_i y_j \mathbf{x}_i \cdot \mathbf{x}_j \quad (3.16)$$

with respect to α_i , subject to the constraints

$$\sum_{i=1}^l \alpha_i y_i = 0 \quad (3.17)$$

$$\alpha_i \geq 0, \quad i = 1, \dots, l \quad (3.18)$$

The optimal separating hyperplane is found by solving the quadratic programming problem, L_D subject to the inequality constraints 3.17 and 3.18. In the solution, the points that lie on H_1 and H_2 with $\alpha_i > 0$ are called *support vectors*. Support vectors satisfy the constraints 3.9 and lie closest to the separating hyperplane. For the remaining points, $\alpha_i = 0$, therefore if these points were removed from the training datasets the optimal separating hyperplane would not change.

The decision function is

$$f(\mathbf{x}) = \text{sign}\left(\sum_{i=1}^l \alpha_i y_i (\mathbf{x} \cdot \mathbf{x}_i)\right) \quad (3.19)$$

where $f(\mathbf{x}) > 0$ means that $\mathbf{x}$ is classified to the positive class, otherwise $\mathbf{x}$ is classified to the negative class.

Linear Non-separable SVM

In most of real life classification problems, the training data points are not linearly separable (Figure 3.8), therefore "soft margin classifiers" are introduced.

Figure 3.8: Linear separating hyperplanes for non-separable SVM. (Adopted from [10])

In this case, linear SVM is extended to non-separable SVM by introducing slack variables, $\xi_i, i = 1, \dots, l$ to relax the hard-margin constraints in Equations 3.7 and 3.8 into :

$$\mathbf{x}_i \cdot \mathbf{w} + b \geq +1 - \xi_i \quad \text{for } y_i = +1 \quad (3.20)$$

$$\mathbf{x}_i \cdot \mathbf{w} + b \leq -1 + \xi_i \quad \text{for } y_i = -1 \quad (3.21)$$

with an additional constraint

$$\xi_i \geq 0 \quad \forall i \quad (3.22)$$

The values of ξ_i indicate which side of the separating hyperplane, the training data point, $\mathbf{x}_i$ lies. $\xi_i \geq 0$, if $\mathbf{x}_i$ is misclassified and lies on the wrong side of the hyperplane, therefore

$\sum_{i=1}^l \xi_i$ is an upper bound on the number of training errors. $\xi_i = 0$, if $\mathbf{x}_i$ is classified correctly, lies on or outside the correct margin. $0 < \xi_i < 1$, if $\mathbf{x}_i$ is classified correctly, but lies inside the margin.

The Lagrange formulation of the minimization problem becomes

$$\min_{\mathbf{w}, b, \xi} L_P \equiv \frac{1}{2} \|\mathbf{w}\|^2 + C \sum_{i=1}^l \xi_i \quad (3.23)$$

where C is a regularization parameter used to control the relation between the slack variables and $\|\mathbf{w}\|$.

The problem is transformed into its dual optimization problem of maximizing L_D ,

$$\max_{\alpha} L_D = \sum_i \alpha_i - \frac{1}{2} \sum_{i,j=1}^l \alpha_i \alpha_j y_i y_j \mathbf{x}_i \mathbf{x}_j \quad (3.24)$$

with respect to α_i , subject to the constraints

$$0 \leq \alpha_i \leq C \quad (3.25)$$

$$\sum_{i=1}^l \alpha_i y_i = 0 \quad (3.26)$$

The difference from the linear separable case is the upper bound C on the Lagrange multipliers α_i . The support vectors are training data points that lie on the separating hyperplane, as well as the data points between the two hyperplanes H_1 and H_2 or data points that lie on the wrong side of the separating hyperplane.

3.2.2.2 Nonlinear Support Vector Machines

In some cases, the decision boundary between the positive and negative training data is not linear. Thus, the input data is mapped into a higher dimensional feature space where it is linearly separable through a nonlinear function

$$\Phi : \mathcal{R}^d \rightarrow \mathcal{H} \quad (3.27)$$

To overcome the problem of finding optimal separating hyperplane in the higher dimensional feature space, the "kernel trick" can be used. The dual Lagrange formulation in Equation

3.24, contains the training data points in the form of Euclidean dot products $\mathbf{x}_i \cdot \mathbf{x}_j$. The dot product can be substituted into the higher dimensional feature space by

$$\mathbf{x}_i \cdot \mathbf{x}_j \rightarrow \Phi(\mathbf{x}_i) \cdot \Phi(\mathbf{x}_j) \quad (3.28)$$

Instead of calculating this mapping explicitly, the function $K(\mathbf{x}_i, \mathbf{x}_j) = \Phi(\mathbf{x}_i) \cdot \Phi(\mathbf{x}_j)$, called kernel function can be used.

Now, the optimization problem in Equation 3.24 becomes L_D ,

$$\max_{\alpha} L_D = \sum_i \alpha_i - \frac{1}{2} \sum_{i,j=1}^l \alpha_i \alpha_j y_i y_j K(\mathbf{x}_i, \mathbf{x}_j) \quad (3.29)$$

subject to the same constraints

$$0 \leq \alpha_i \leq C \quad (3.30)$$

$$\sum_{i=1}^l \alpha_i y_i = 0 \quad (3.31)$$

When the optimization problem is solved, the decision function becomes

$$f(\mathbf{x}) = \text{sign}\left(\sum_{i=1}^l \alpha_i y_i K(\mathbf{x}, \mathbf{x}_i)\right) \quad (3.32)$$

where $f(\mathbf{x}) > 0$ means that $\mathbf{x}$ is classified to the positive class, otherwise $\mathbf{x}$ is classified to the negative class.

Some commonly used kernel functions are shown in Table 3.4.

Table 3.4: Kernel functions used for nonlinear SVMs.

Linear	$K(\mathbf{x}_i, \mathbf{x}_j) = (\mathbf{x}_i \cdot \mathbf{x}_j)$
Polynomial	$K(\mathbf{x}_i, \mathbf{x}_j) = (\gamma \mathbf{x}_i \cdot \mathbf{x}_j + r)^d$
RBF	$K(\mathbf{x}_i, \mathbf{x}_j) = e^{\frac{-\gamma \ \mathbf{x}_i - \mathbf{x}_j\ ^2}{\sigma^2}}$
Sigmoid	$K(\mathbf{x}_i, \mathbf{x}_j) = \tanh(\gamma \mathbf{x}_i \cdot \mathbf{x}_j + r)$

CHAPTER 4

RESULTS AND DISCUSSION

In this study a discriminative classification system is applied to the first-, second- and third-level Enzyme Commission classes defined by IUBMB. The hierarchical tree structure is used to extract representative enzymes for positive and negative training datasets.

Five-fold cross validation is used to evaluate the performance of the system. In five-fold cross validation, the dataset is divided into five folds, four folds are used to train the classifier and one fold is used to test the classifier. The classification is repeated five times so that each fold is used for testing once. In this study, instead of dividing the dataset into five folds randomly, we added representative samples from all sub-sub-subclasses in the EC tree to each fold.

Sensitivity, specificity and area under the ROC (receiver operating characteristic) curve values are used to evaluate the statistical significance of the classification system.

In the binary classification case, the classifier maps the samples to either positive or negative class. The correct and incorrect test results of a classifier can be displayed in a two-by-two *confusion matrix* as shown in Table 4.1.

Table 4.1: Confusion Matrix

		Predicted class	
		Positive	Negative
True class	Positive	True Positive	False Negative
	Negative	False Positive	True Negative

Sensitivity (Equation 4.1) is the probability of correctly classified positive enzymes among all positive enzymes in the test dataset.

$$sensitivity = \frac{True\ positives}{True\ positives + False\ negatives} \quad (4.1)$$

Specificity (Equation 4.2) is the probability of correctly classified negative enzymes among all negative enzymes in the test dataset.

$$specificity = \frac{True\ negatives}{True\ negatives + False\ positives} \quad (4.2)$$

Area under the ROC (receiver operating characteristic) curve represents the probability that the classifier will rank a randomly chosen positive sample higher than a randomly chosen negative sample [9].

4.1 First-Level EC Classification Results

Classification is performed among six major EC classes at the first-level. Table 4.2 shows average five-fold cross validation results for classification of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6), respectively. The results are promising to continue the classification task at the second-level.

The average sensitivity values for oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6) are 92.08%, 93.38%, 92.84%, 91.24%, 91.76% and 97.16%, respectively.

The average specificity values for oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6) are 99.20%, 96.86%, 97.92%, 99.90%, 99.94% and 99.90%, respectively.

The average AUC values for oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5) and ligases (EC 6) are 0.992, 0.989, 0.991, 0.992, 0.994 and 0.998, respectively.

We didn't decompose the results for all five folds, but the results are very close to each other

for all five folds, demonstrating the positive and negative datasets represent the classes well.

Table 4.2: Average Five-fold Cross Validation Results for the First-Level EC Classes , TP : True Positives , FP : False Positives , TN : True Negatives , FN : False Negatives , sens. : sensitivity , spec. : specificity , AUC : Area under the ROC curve.

EC Class	TP	FP	TN	FN	sens. (%)	spec. (%)	AUC
EC 1 Oxidoreductases	18255	323	39807	1567	92.08	99.20	0.992
EC 2 Transferases	28155	993	30601	1990	93.38	96.86	0.989
EC 3 Hydrolases	24766	780	36933	1907	92.84	97.92	0.991
EC 4 Lyases	12229	25	43421	1166	91.24	99.90	0.992
EC 5 Isomerases	7055	6	45663	631	91.76	99.94	0.994
EC 6 Ligases	17470	40	42372	511	97.16	99.90	0.998

4.2 Second-Level EC Classification Results

In the second level, 57 sub-classes which have more than 25 enzymes are selected for classification among 60 sub-classes. Table 4.3 shows average five-fold cross validation results for second-level EC sub-classes. The average sensitivity values are in the range 83.08% to 100% except sub-classes EC 1.12 and EC 3.8. Table 4.4 and Table 4.5 show the five-fold cross-validation results for EC 1.12 and EC 3.8. The low average sensitivity value, 64.28% for EC 1.12 is because of the unbalanced distribution of the datasets where there are 56 enzymes in each fold of the positive dataset and about 16918 enzymes in each fold of the negative dataset for this sub-class. Sub-class EC 3.8 has 76.66% average sensitivity because of the same reason. The average specificity values are over 98% for all sub-classes demonstrating that the negative datasets have enough number of representative enzymes. Although the datasets are different, if we compare the accuracy values, our results are better than the second-level

classification results presented in Chapter 2, establishing that our training datasets are well identified to discriminate the positive and negative classes.

Table 4.3: Average Five-fold Cross Validation Results for the Second-Level EC Classes , TP : True Positives , FP : False Positives , TN : True Negatives , FN : False Negatives , sens. : sensitivity , spec. : specificity , AUC : Area under the ROC curve.

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 1.1	5237	17	14778	235	95.70	99.88	0.997
EC 1.2	2143	0	17995	122	94.58	100	0.998
EC 1.3	1287	1	18833	136	90.42	99.98	0.995
EC 1.4	995	0	19208	65	93.84	100	0.998
EC 1.5	844	0	19323	110	88.44	100	0.991
EC 1.6	2924	5	17231	89	97.04	99.92	0.998
EC 1.7	636	0	19567	43	93.68	100	0.998
EC 1.8	833	0	19389	50	94.34	100	0.997
EC 1.9	486	0	19647	99	83.08	100	0.995
EC 1.10	162	0	20089	8	95.30	100	0.998
EC 1.11	692	0	19496	68	91.06	100	0.994
EC 1.12	45	0	20204	25	64.28	100	0.990
EC 1.13	329	0	19907	36	90.08	100	0.993
EC 1.14	880	0	19276	106	89.26	100	0.995
EC 1.15	395	0	19838	25	94.04	100	0.996
EC 1.16	85	0	20264	10	89.48	100	0.999
EC 1.17	749	0	19457	37	95.30	100	0.995
EC 1.18	178	0	20071	22	89.00	100	0.996
EC 1.20	9	0	20231	1	90.00	100	1.000
EC 1.21	19	0	20242	2	92.00	100	1.000
EC 1.97	48	0	20205	2	96.00	100	1.000
EC 2.1	6015	12	38812	421	93.44	99.90	0.997

Table 4.3 - Continued

EC Class	TP	FP	TN	FN	sens. (%)	spec. (%)	AUC
EC 2.2	996	0	44275	18	98.20	100	0.999
EC 2.3	3100	5	41694	397	88.66	99.96	0.994
EC 2.4	5093	14	39775	373	93.20	99.92	0.996
EC 2.5	3595	3	41352	321	91.78	99.94	0.996
EC 2.6	1399	0	43856	57	96.08	100	0.999
EC 2.7	21864	277	22443	575	97.46	98.78	0.998
EC 2.8	803	0	44439	57	93.36	100	0.998
EC 2.9	80	0	45213	0	100	100	1.000
EC 3.1	7928	155	18516	694	91.98	99.16	0.994
EC 3.2	1960	23	24951	366	84.28	99.90	0.993
EC 3.3	231	0	27041	9	96.24	100	0.999
EC 3.4	3801	12	23105	363	91.28	99.90	0.994
EC 3.5	5044	6	21870	389	92.82	99.94	0.994
EC 3.6	6110	8	20947	203	96.78	99.94	0.998
EC 3.7	82	0	27218	3	96.46	100	1.000
EC 3.8	23	0	27292	7	76.66	100	1.000
EC 3.11	38	0	27252	2	95.00	100	1.000
EC 4.1	4719	20	8893	153	96.86	99.76	0.999
EC 4.2	6568	13	7077	134	98.00	99.84	0.999
EC 4.3	867	0	12872	41	95.50	100	0.996
EC 4.4	334	0	13434	15	95.70	100	0.999
EC 4.6	408	0	13277	12	97.66	100	0.999
EC 4.99	432	0	13338	13	97.10	100	0.998
EC 5.1	1211	1	6533	32	97.42	99.98	0.998
EC 5.2	638	0	5822	47	93.12	100	0.999
EC 5.3	3051	9	4656	58	98.14	99.82	0.999
EC 5.4	2212	0	5517	62	97.28	100	0.998
EC 5.5	80	0	7705	5	94.10	100	0.990
EC 5.99	361	1	7406	24	93.76	99.98	0.999

Table 4.3 - Continued

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 6.1	9479	11	7569	18	99.76	99.82	0.999
EC 6.2	746	0	17264	14	98.12	100	0.999
EC 6.3	6359	11	11590	56	99.08	99.90	0.999
EC 6.4	610	0	17398	7	98.84	100	0.999
EC 6.5	724	0	17276	16	97.76	100	0.998
EC 6.6	30	0	17985	5	85.70	100	0.993

Table 4.4: Five-fold Cross Validation Results for EC 1.12 , TP : True Positives , FN : False Negatives , TN : True Negatives , FP : False Positives , sens. : sensitivity , spec. : specificity , AUC : Area under the ROC curve.

EC 1.12									
	Positive Training	Negative Training	Positive Test		Negative Test		sens.	spec.	AUC
			tp	fn	tn	fp			
Fold 1	56	16921	9	5	4034	0	0.643	1.0	0.972
Fold 2	56	16921	9	5	4063	0	0.643	1.0	1.0
Fold 3	56	16914	10	4	4041	0	0.714	1.0	0.999
Fold 4	56	16922	6	8	4027	0	0.429	1.0	0.986
Fold 5	56	16916	11	3	4039	0	0.785	1.0	0.995

Table 4.5: Five-fold Cross Validation Results for EC 3.8 , TP : True Positives , FN : False Negatives , TN : True Negatives , FP : False Positives , sens. : sensitivity , spec. : specificity , AUC : Area under the ROC curve.

EC 3.8									
	Positive Training	Negative Training	Positive Test		Negative Test		sens.	spec.	AUC
			tp	fn	tn	fp	(%)	(%)	(%)
Fold 1	28	22614	6	0	5449	0	100	100	100
Fold 2	28	22616	5	1	5470	0	83,3	100	100
Fold 3	28	22615	4	2	5468	0	66,7	100	100
Fold 4	28	22611	5	1	5449	0	83,3	100	100
Fold 5	28	22626	3	3	5456	0	50	100	100

4.3 Third-Level EC Classification Results

The classification is performed by 140 sub-subclasses shown in Table 4.6, which have more than 25 enzymes at the third-level. The sub-classes of EC 1.9, EC 1.11, EC 1.15, EC 1.97, EC 2.2, EC 2.5, EC 2.9, EC 3.7, EC 3.8, EC 3.11, EC 4.4, EC 4.6, EC 4.99, EC 5.2, EC 5.5, EC 5.99, EC 6.1, EC 6.2, EC 6.4, EC 6.5 and EC 6.6 have only one sub-subclass at the third level, therefore the classification at the second-level would be sufficient to assign a novel enzyme to these sub-subclasses. The predictive results for the third-level classifications are good for most of the sub-subclasses. Among 140 sub-subclasses, the average sensitivity values of 11 sub-subclasses are less than 80.00%. These sub-subclasses are EC 1.1.3, EC 1.2.7, EC 1.2.99, 1.3.99, EC 1.5.3, EC 1.7.3, EC 1.8.3, EC 1.16.1, EC 1.18.1, EC 3.1.21, EC 3.4.16. There are very small number of enzymes in the positive training datasets of these sub-subclasses. When the sensitivity values of each fold of the sub-subclasses having small number of positive samples are observed, it is seen that sensitivity values are very high for some folds and very low for some other folds. But when we look at the classes with sufficient number of training samples, the results for all folds are consistent and high. The average specificity values for all sub-subclasses are over 95.00% except EC 1.5.1 and EC 1.13.11, since there are sufficient number of negative training samples for most of the sub-subclasses. The number of negative samples in the datasets of EC 1.5.1 and EC 1.13.11 are few and less than the number of positive samples, therefore average specificity values of these sub-subclasses are less than the remaining sub-subclasses. Average AUC values are over 0.95 for all of the sub-subclasses.

Table 4.6: Average Five-fold Cross Validation Results for the Third-Level EC Classes , TP : True Positives , FP : False Positives , TN : True Negatives , FN : False Negatives , sens. : sensitivity , spec. : specificity , AUC : Area under the ROC curve.

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 1.1.1	5295	24	454	0	100	95.10	0.998
EC 1.1.2	59	0	5707	6	90.96	100	1.000

Table 4.6 - Continued

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 1.1.3	38	0	5724	16	77.62	100	0.996
EC 1.1.99	328	0	5423	23	93.72	100	0.996
EC 1.2.1	2016	2	340	14	99.30	99.48	0.999
EC 1.2.4	193	0	2167	12	94.18	100	0.998
EC 1.2.7	35	0	2309	30	54.12	100	0.999
EC 1.2.99	25	0	2326	24	51.84	100	0.994
EC 1.3.1	698	0	839	30	96.04	100	0.998
EC 1.3.3	592	0	958	16	97.28	100	0.999
EC 1.3.5	41	0	1521	5	89.12	100	1.000
EC 1.3.7	46	0	1515	4	92.88	100	0.999
EC 1.3.99	103	0	1431	32	77.42	100	0.986
EC 1.4.1	166	0	938	17	91.16	100	0.999
EC 1.4.3	410	2	695	14	96.70	99.70	0.999
EC 1.4.4	382	0	739	0	100	100	1.000
EC 1.4.99	114	0	1000	7	94.30	100	0.999
EC 1.5.1	929	9	81	4	99.60	89.66	0.991
EC 1.5.3	9	0	998	16	34.66	100	0.973
EC 1.5.99	41	0	970	9	83.00	100	0.998
EC 1.6.1	76	0	2953	4	94.82	100	1.000
EC 1.6.2	106	0	2929	1	99.04	100	1.000
EC 1.6.5	1240	26	1705	41	96.80	98.50	0.997
EC 1.6.99	1490	6	1471	45	97.08	99.62	0.998
EC 1.7.1	466	1	239	7	98.54	99.54	0.999
EC 1.7.2	72	0	639	0	100	100	1.000
EC 1.7.3	20	0	682	9	67.62	100	0.972
EC 1.7.99	121	0	582	8	93.66	100	0.999
EC 1.8.1	397	0	518	3	99.26	100	0.999
EC 1.8.3	28	0	879	10	73.42	100	0.998
EC 1.8.4	430	0	485	2	99.54	100	1.000

Table 4.6 - Continued

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 1.8.98	28	0	884	5	85.22	100	1.000
EC 1.10.2	37	0	151	2	94.64	100	0.999
EC 1.10.3	91	0	97	2	97.88	100	1.000
EC 1.10.99	58	0	132	0	100	100	1.000
EC 1.12.99	31	0	48	4	88.56	100	0.984
EC 1.13.11	394	6	27	0	100	85.50	0.998
EC 1.14.11	101	0	1046	23	81.72	100	0.998
EC 1.14.12	74	0	1092	9	90.54	100	0.990
EC 1.14.13	217	0	897	61	80.48	100	0.982
EC 1.14.14	296	8	854	20	93.66	99.04	0.998
EC 1.14.15	35	4	1125	7	86.88	99.62	0.977
EC 1.14.16	31	0	1145	3	90.00	100	1.000
EC 1.14.17	35	0	1138	4	89.16	100	1.000
EC 1.14.18	33	0	1137	8	80.56	100	0.999
EC 1.14.19	31	0	1146	3	91.10	100	0.997
EC 1.14.99	166	0	997	9	95.18	100	0.993
EC 1.16.1	17	0	84	9	67.68	100	0.997
EC 1.16.3	78	0	27	5	93.90	100	0.992
EC 1.17.1	312	0	479	10	96.94	100	0.999
EC 1.17.4	471	2	327	1	99.78	99.42	1.000
EC 1.18.1	53	0	141	14	79.56	100	0.999
EC 1.18.6	138	1	66	3	97.84	98.46	0.996
EC 2.1.1	3832	16	2879	8	99.78	99.44	0.999
EC 2.1.2	2006	0	4721	8	99.62	100	0.999
EC 2.1.3	863	0	5865	7	99.20	100	0.998
EC 2.3.1	3343	27	797	6	99.82	96.74	0.998
EC 2.3.2	418	0	3742	19	95.70	100	0.999
EC 2.3.3	375	3	3782	12	96.94	99.94	0.999
EC 2.4.1	2274	20	3380	21	99.12	99.42	0.999

Table 4.6 - Continued

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 2.4.2	3320	4	2327	43	98.74	99.84	0.999
EC 2.4.99	38	0	5659	0	100	100	1.000
EC 2.6.1	1293	0	198	0	100	100	1.000
EC 2.6.99	189	0	1299	0	100	100	1.000
EC 2.7.1	4795	19	18265	255	94.26	99.90	0.997
EC 2.7.2	1647	0	21725	23	98.62	100	0.999
EC 2.7.3	99	0	19284	9	91.74	100	0.999
EC 2.7.4	2908	2	20420	64	97.84	99.96	0.998
EC 2.7.6	252	2	23130	11	95.90	99.98	0.995
EC 2.7.7	8327	83	14743	214	97.52	99.44	0.999
EC 2.7.8	1049	2	22291	40	96.34	99.98	0.996
EC 2.7.9	119	0	23255	6	95.36	100	1.000
EC 2.7.10	440	0	22904	32	93.22	100	0.998
EC 2.7.11	2378	39	20873	105	95.80	99.82	0.999
EC 2.7.12	87	0	23280	19	82.04	100	0.999
EC 2.7.13	412	0	22935	27	93.84	100	0.999
EC 2.8.1	808	0	204	9	98.74	100	0.999
EC 2.8.2	98	0	828	4	96.80	100	0.999
EC 2.8.3	61	0	864	5	92.90	100	0.999
EC 2.8.4	30	0	894	6	83.92	100	0.999
EC 3.1.1	1696	15	7172	113	93.80	99.78	0.997
EC 3.1.2	450	0	8514	36	92.60	100	0.992
EC 3.1.3	1830	7	6987	191	90.56	99.90	0.996
EC 3.1.4	506	0	8407	92	84.58	100	0.995
EC 3.1.5	30	0	8975	1	96.66	100	1.000
EC 3.1.6	27	0	8973	6	80.84	100	0.968
EC 3.1.11	602	0	8360	36	94.40	100	0.997
EC 3.1.13	104	0	8889	16	86.92	100	0.997
EC 3.1.21	271	0	8614	118	69.68	100	0.972

Table 4.6 - Continued

EC Class	TP	FP	TN	FN	sens.	spec.	AUC
					(%)	(%)	
EC 3.1.22	408	0	8598	7	98.32	100	0.998
EC 3.1.26	2139	0	6768	87	96.08	100	0.998
EC 3.1.27	192	0	8793	21	90.30	100	0.999
EC 3.2.1	1882	25	582	7	99.62	95.94	0.999
EC 3.2.2	561	0	1890	46	92.46	100	0.997
EC 3.3.1	192	0	62	1	99.50	100	0.999
EC 3.3.2	58	0	193	4	93.76	100	0.999
EC 3.4.11	574	0	4229	43	93.08	100	0.995
EC 3.4.13	131	0	4696	14	90.36	100	0.994
EC 3.4.14	79	0	4754	9	89.50	100	0.999
EC 3.4.16	25	0	4796	19	56.00	100	0.968
EC 3.4.17	59	0	4770	10	87.32	100	0.980
EC 3.4.19	141	0	4688	23	86.08	100	0.993
EC 3.4.21	1614	3	3165	60	96.58	99.90	0.998
EC 3.4.22	280	0	4526	35	90.14	100	0.998
EC 3.4.23	482	0	4342	19	96.42	100	0.999
EC 3.4.24	894	0	3924	24	97.52	100	0.999
EC 3.4.25	272	0	4561	10	96.44	100	0.999
EC 3.5.1	1724	11	3877	100	94.62	99.72	0.998
EC 3.5.2	609	0	5074	31	95.16	100	0.996
EC 3.5.3	505	0	5197	11	97.92	100	0.999
EC 3.5.4	2416	0	3244	52	97.92	100	0.999
EC 3.5.99	244	0	5461	8	96.92	100	0.997
EC 3.6.1	2562	6	4096	15	99.40	99.86	0.999
EC 3.6.3	4041	4	2624	14	99.64	99.84	0.999
EC 3.6.4	25	0	6655	2	92.00	100	1.000
EC 4.1.1	3767	31	1201	22	99.44	97.54	0.999
EC 4.1.2	569	0	4412	44	92.90	100	0.997
EC 4.1.3	362	0	4629	26	93.34	100	0.996

Table 4.6 - Continued

EC Class	TP	FP	TN	FN	sens. (%)	spec. (%)	AUC
EC 4.1.99	219	0	4792	10	95.60	100	0.999
EC 4.2.1	5329	30	1494	11	99.78	98.06	0.999
EC 4.2.2	78	0	6765	21	81.76	100	0.999
EC 4.2.3	917	0	5917	31	96.76	100	0.999
EC 4.2.99	461	0	6388	16	96.66	100	0.999
EC 4.3.1	418	2	512	8	98.18	99.60	0.999
EC 4.3.2	504	0	431	5	99.00	100	1.000
EC 5.1.1	794	0	567	8	99.02	100	0.999
EC 5.1.2	137	0	1233	1	99.32	100	0.998
EC 5.1.3	409	1	946	14	96.86	99.90	0.999
EC 5.3.1	2768	4	484	8	99.72	99.16	0.999
EC 5.3.3	340	0	2903	19	94.74	100	0.998
EC 5.3.4	63	0	3195	4	93.84	100	1.000
EC 5.3.99	46	0	3213	4	92.24	100	1.000
EC 5.4.2	1393	0	912	13	99.06	100	0.999
EC 5.4.3	368	0	1946	3	99.20	100	0.999
EC 5.4.99	502	0	1796	19	96.34	100	0.999
EC 6.3.1	437	0	6043	19	95.90	100	0.999
EC 6.3.2	2656	1	3833	15	99.42	99.98	0.999
EC 6.3.3	510	0	5980	10	98.08	100	0.998
EC 6.3.4	1704	0	4779	16	99.06	100	0.999
EC 6.3.5	1118	0	5366	14	98.76	100	0.999

4.4 Discussion

In this thesis, we used a top-down approach for prediction of Enzyme Commission classes. Since we performed five-fold cross-validation, we prepared 1015 positive and 1015 negative datasets for 203 classes. We developed an algorithm to select representative samples from the hierarchical tree structure for the positive and negative datasets of a class, to improve the performance of the classification model we used. We trained 30, 285, 700 classifiers for the first-, second- and third-level classes, respectively. Average sensitivity, specificity and AUC results are very good for the first-level classes as we expected, because the number of positive and negative samples belonging to the classes at this level are more than enough to make an accurate distinction. The results are also good for the second-level subclasses, except for only two subclasses. At the third-level the prediction results are better than expected, since the number of positive samples belonging to sub-subclasses at this level are very few compared to the higher levels.

CHAPTER 5

CONCLUSION AND FUTURE WORK

In this thesis, we developed a three-level classification system for the prediction of enzyme classes. Our emphasis in this thesis was on the selection of positive and negative enzymes for training datasets to improve the predictive performance of the discriminative model. We developed an algorithm to prepare positive and negative datasets for the classes represented in the four-level hierarchical tree structure by IUBMB. We used a subsequence-based approach, Subsequence Profile Map (SPMap), to generate features from primary sequences of enzymes. The results were satisfactory for the first- and second-level classes, as well as most of the classes at the third-level.

For future work, classification between enzymes and non-enzymes can be performed by using the same algorithm developed in this study to extract the enzymes from the hierarchical tree structure and developing an algorithm to select representative non-enzyme proteins.

In this study, negative enzymes are selected from the classes having the same parent as the positive class, at the higher level. For two EC numbers EC $x_1.x_2.x_3.x_4$ and EC $y_1.y_2.y_3.y_4$, if $x_1 = y_1$ and $x_2 \neq y_2$, the enzymes belonging to these numbers catalyze the same reactions at the first-level, but the type of the substrate is different at the second-level. For our case, to give an example, for the EC class, EC 1.1, negative examples are selected from the remaining subclasses EC 1. x , $x = 2, \dots, 97$. For further consideration, since same digits representing the level in the EC number, refers to the same function, negative enzymes can be selected from the classes with different parents at the higher level, but with same function at the same level. For the same EC class, EC 1.1, the negative enzymes can be selected from EC $y.1$, $y = 2, \dots, 6$. Thus, negative samples may be far away from the positive samples in the feature space.

SPMap was used to classify the enzyme datasets prepared by Lu et al. [29] and the results

were presented in [35]. These datasets were constructed by removing proteins having more than 25% sequence identity. Since we claim that our datasets are more discriminative, SPMMap method can be compared by other feature extraction methods using the datasets we prepared in this study. Amino acid composition of enzymes can be used to generate feature vectors and SVMs can be used for classification. The results can be compared with the results obtained in this study.

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