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Prediction of Enzyme Class

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摘要

酵素，是催化劑的一種，因其中的化學反應及功能不同，而分成六類．與利用實驗得知相比，利用結構來預測蛋白質功能的方法日益重要．這篇論文中，我們描述了一些以序列及結構為基礎的編碼系統．我們使用不同的編碼系統在兩個方法上，一個是兩階式支持向量機器方法，另一個則是這篇文章中所描述的霍夫曼樹模型的方法．這個利用支持向量機器的霍夫曼樹模型被提供對未知功能的酵素，預測其酵素分類．比較了兩個方法，使用霍夫曼樹模型我們可以得到一個沒有偏倚而且最好可達 36%的準確率，這也證實霍夫曼樹模型在酵素分類的預測上是有用的．

Prediction of Enzyme Class

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ABSTRACT

Enzymes, as a subclass of catalysts, can be separated into six parts since they have different chemical reactions and protein functions. Methods for predicting protein function from structure are becoming more important than experimental knowledge. In this study, we describe some coding schemes which include both sequence-based and structure-based protein information. We predict the enzyme class for different coding schemes with 2 methods; one is the 2-level SVM model method, one is the Huffman tree model method which is described in this study. This Huffman tree model using support vector machine (SVM) is provided to predict the enzyme classification from the unknown- function enzymes. By comparing with these methods, Huffman tree model is demonstrated useful on enzyme class predicting since we can obtain unbiased and the best prediction accuracy of 36% using the Huffman tree model.

誌謝

在研究所的兩年時間，我學到了很多，不管是課業方面，還是待人接物方面，更重要的是一個人的態度．在做研究的時候就應該極力鑽研，秉持打破沙鍋問到底的原則，將事物作透徹的分析；在休閒的時候，就要懂得適當地放鬆心情，不要一心二用，反而無法兩者兼顧．

多謝我的指導老師黃鎮剛老師，老師並不光是指派任務讓我們完成，而是讓我們在這個任務當中得到一些新的啟發，再經由這些啟發給予適當的建議，適當地指引方向．也由於老師的教導方式，讓我知道思考在研究當中是最重要的一環．

另外還要感謝實驗室的所有同伴，在我有困難的時候給了我很多的幫助，讓我知道了許多問題都發生在之前沒有想到會成為問題的地方．謝謝陸志豪學長不僅在研究上提攜我，還找我一起打球運動．謝謝游景盛學長在一開始就先教我ＳＶＭ的使用，還在我有問題的時候給我很多協助．謝謝簡思樸，盧慧及葉書瑋同學在這兩年內彼此鼓勵，互相學習，還有最後的時候互相盯對方的進度．另外也多謝其他實驗室的學長姐們．

CONTENTS

INTRODUCTION

Catalysts, generally speaking, are specific in nature as to the type of reaction they can catalyze. Enzymes, as a subclass of catalysts, are very specific in nature. Each enzyme can act to catalyze only very select chemical reactions and only with very select substances. An enzyme has been described as a "key" which can "unlock" complex compounds. An enzyme, as the key, must have a certain structure or multi-dimensional shape that matches a specific section of the "substrate" (a substrate is the compound or substance which undergoes the change). Once these two components come together, certain chemical bonds within the substrate molecule change much as a lock is released, and just like the key in this illustration, the enzyme is free to execute its duty once again.

The Enzyme Commission number (EC number) is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze. As a system of enzyme nomenclature, every EC number is associated with a recommended name for the respective enzyme.

Every enzyme code consists of the letters "EC" followed by four numbers separated by periods. Those numbers represent a progressively finer classification of the enzyme. For example, the enzyme tripeptide aminopeptidase has the code "EC 3.4.11.4", whose components indicate the following groups of enzymes: EC 3 enzymes are hydrolases (enzymes that use water to break up some other molecule), EC 3.4 are hydrolases that act on peptide bonds, EC 3.4.11 enzymes are only those hydrolases that cleave off the amino-terminal amino acid from a polypeptide, and EC 3.4.11.4 are those that cleave off the amino-terminal end from a tripeptide.

Strictly speaking, EC numbers do not specify enzymes, but enzyme-catalyzed reactions. If different enzymes (for instance from different organisms) catalyze the

same reaction, then they receive the same EC number. UniProt identifiers uniquely specify a protein by its amino acid sequence. Here are some brief introduction of six classes of enzymes in Appendix.

The enzyme nomenclature scheme was developed starting in 1955, when the International Congress of Biochemistry in Brussels set up an Enzyme Commission. The first version was published in 1961. The current sixth edition, published by the International Union of Biochemistry and Molecular Biology in 1992, contains 3196 different enzymes.

Here are some related numbers such as the number of each enzyme class or the number of subclass in each enzyme class listed in Table 1. There are more and more unknown function proteins found in recent years (see Figure 1). To know the classification of these enzymes is important; we can know what reactions they will do, what kinds of catalytic sites they are, etc. However, enzymes are classified into six classes by experimental supports so far and it takes a lot of time. If an enzyme can be classified in computational way, it is faster, cheaper, and simpler to recognize the enzyme class in the future.

Simulating the molecular and atomic mechanisms that define the function of a protein is beyond the current knowledge of biochemistry and the capacity of available computational power. Similarity search among proteins with known function is consequently the basis of current function prediction (Whisstock and Lesk 2003). A newly discovered protein is predicted to exert the same function as the most similar proteins in a database of known proteins. This similarity among proteins can be defined in a multitude of ways: two proteins can be regarded to be similar, if their sequences align well [e.g. PSI-BLAST (Altschul, Madden et al. 1997)], if their structures match well [e.g. DALI (Holm and Sander 1996)], if both have common

surface clefts or bindings sites [e.g. CASTp (Binkowski, Naghibzadeh et al. 2003)], similar chemical features or common interaction partners [e.g. DIP (Xenarios, Salwinski et al. 2002)], or if both contain certain motifs of amino acids (AAs) [e.g. Evolutionary Trace (Yao, Kristensen et al. 2003)]. An armada of protein function prediction systems that measure protein similarity by one of the conditions above has been developed. Each of these conditions is based on a biological hypothesis; e.g. structural similarity implies that two proteins could share a common ancestor and that they both could perform the same function as this common ancestor (Bartlett *et al*., 2003).

We can take oxidoreductases for example. Here are six structures of some enzymes known as oxidoreductases (EC No. 1) in Figure 2. All these enzymes are classified as oxidoreductases, but they have low structure and sequence similarities with each other which are shown in Table 2, and three of these proteins are even in the same subclass. Since there are no same characteristics found in structures and sequences, we want to check if it is possible to have characteristics in mechanisms. We take three proteins for examples which are classified to the same subclass in Figure 2 to see their mechanisms in the reactions. Here are the reactions of these proteins in Table 3. We can also see the differences in other enzyme class such as in Figure 3 and Figure 4. Methods mentioned above are not useful enough for predicting enzyme class. Because of the diversity of protein structures and mechanisms, we can not use DALI or CASTp to predict enzyme functions. Because of the low sequence similarity between 2 proteins, using PSI-BLAST may not be reliable.

There are some methods to predict enzyme class. Dobson and Doig provided a 2-level SVMs model and the features they used include protein composition, surface protein composition, secondary structures, general protein information, and some metal atoms. They use not only sequence but also structure-based information as

SVM features. In their paper, they can get a 35% accuracy of prediction, but that is not good enough. Here we provide a Huffman tree model in order to get higher accuracy.

DATASET

498 enzymes (Dobson and Doig 2005) have been chosen using function definitions obtained from DBGet (Fujibuchi, Goto et al. 1997) PDB Enzyme (Bairoch 2000) cross-links and structural relations from the Astral SCOP 1.63 superfamily level dataset. The Astral lists were culled so that only whole protein structures with a SPACI score (Brenner, Koehl et al. 2000) of 0.3 or greater could be selected for each functional class. The distribution of each enzyme classification is listed in Table 4. All proteins used in this work are listed in Appendix.

These 498 enzymes are taken to do pair wise multiple sequence alignments (MSA) and almost 100% of alignments have less than 20% sequence identity. In each functional class no structure contains a domain from the same superfamily as any other structure.

METHODS

In Figure 5, here is the flow chart which we need to individually introduce every state in order.

Coding schemes

We use both structure-based and sequence-based protein information as our SVM features.

At structure-based protein information part, there is a website http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/CSS/makeEbiHtml.cgi?file=form.html which is used to provide us some information as structural protein information.

This catalytic site search (CSS) web server allows us to submit protein structures and search them using the related bank of structural templates(Torrance, Bartlett et al. 2005), in order to identify residue patterns resembling known catalytic sites. Structural templates describe small groups of residues within protein structures, such as the Ser-His-Asp catalytic triad in the serine proteases (see Figure 6). Structural templates can be used with a template searching program to search a protein of unknown function for residue patterns whose function is known. This server uses a library of templates describing catalytic sites, derived from the Catalytic Site Atlas (CSA).

The catalytic site atlas is a database describing catalytic sites in enzymes of known structure. The annotations in the CSA are taken manually from the scientific literature. This annotation is extended to related enzymes in the following manner: the sequence similarity searching program PSI-BLAST is used to search for relatives of the enzymes which have been manually annotated; relatives which have conserved catalytic residues are selected; the catalytic residue annotation is transferred from the manually annotated entries to their selected relatives.

Each literature entry together with its annotated relatives constitutes a CSA family. For each member of a given family, a structural template is constructed representing its catalytic residues. An all-against-all superposition is carried out on templates within the family, in order to determine structural distances between all templates, quantified by RMSD/E-value. The template with the lowest RMSD/E-value from all other family members is the representative template for that family. The representative templates for a non-overlapping set of 147 families are used for searching by this server (shown in Appendix).

All templates are capable of matching similar residue types: Glu can match Asp, Gln can match Asn and Ser can match Thr. Additionally, certain equivalent atom types

can match one another. For example, the two oxygens in the carboxyl group of Glu are chemically equivalent to one another, but have different names in PDB files. They are permitted to match either way around.

This server uses the template matching program Jess(Barker and Thornton 2003) to search for matches to structural templates. Jess does not detect every possible combination of residues within a protein that match to a given template. That would take too much time. Instead, Jess only detects those hits where all inter-atom distances are within 6 Å of the equivalent inter-atom distances in the template. Only the best hit that Jess obtains between a given template and a given protein is reported. A summary of all hits obtained by Jess is provided, in order of E-value. An example of the output is shown as Figure 7. Here are the expansions of each column: hit rank column (hits are ranked by their E-values); template column (each template is based upon an entry in the CSA, which corresponds to a single PDB code); description in PDBsum column (a brief description of the protein structure upon which the template is based); RMSD column (root mean square deviation between the template atoms and the matching atoms in the target); E-value column (the E-value is the number of hits of this quality or better that you would expect to obtain at random. E-values are useful because the statistical significance of RMSD values is not equivalent from one template to another); assessment column (an assessment of how likely the hit is to be meaningful. It is based solely on the E-value. E-value < 1e-8: Highly probable; 1e-8 < E-value < 1e-5: Probable; 1e-5 < E-value < 0.1: Possible; E-value > 0.1: Unlikely); details column (which provides a link to further details of the match, given further down the page); residues in template (which describes the residues in the template structure which matched your query); matching residues in query structure (which shows which residues in your query matched which residues in the template); raw

Jess output (At the bottom of the output page there is a link to the raw output produced by Jess(Barker and Thornton 2003)).

Catalytic site atlas (CSA), a resource of catalytic sites and residues identified in enzymes using structural data, provides catalytic residue annotation for enzymes in the Protein Data Bank. In CSA, many 3-D templates are created as specific 3-D conformations of small numbers of residues. The enzyme active-site templates are used in this work. Each of them consists of one, two or three residues that are known from the literature to be catalytic, plus one or more additional residues whose 3-D positions are highly conserved relative to the catalytic residues. It is available online at http://www.ebi.ac.uk/thornton-srv/databases/CSA.

Several different protein compositions is used to generate sequence- based information for getting better results on SVM.

A general global sequence descriptor based on the protein composition coding has been used to discriminate protein properties in a number of applications. The C coding means the usual amino acid composition. The D coding gives the dipeptide composition. We use YX_n to denote the partitioned amino acid composition in which the sequence is partitioned into n subsequences of equal length, and each fragment encoded by the particular amino acid composition Y. For example, the notation CX_5 denotes that the sequence is divided into 5 subsequences, each of which is encoded by C (note that CX_1 is equivalent to C and we use only X_n to substitute CX_n). The coding YX_n provides information about the local properties of sequences.

Another generalized sequence composition is the n-gap dipeptide compositions, denoted by Dj_n, in which we compute the composition of the sequence of the form,

$a(x)_n b$ where a and b denote two specific amino acid types, and $(x)_n$ denotes n intervening amino acids of arbitrary type x. Note that in the special case of $n=0$, Dj_0 is equivalent to D.

In addition, we use $N_l C$ to denote the amino acid composition of a sliding window of length l centered on a given amino acid type. The $N_l C$ provides information of the flanking sequences of a given amino acid type. Note that when l is the length L of the whole sequence, $N_L C$ reduces to C. This coding schemes are list in Table 5.

Besides, we regroup the amino acids into smaller number of classes according to their physico-chemical properties. In this work, we use the following classification schemes of the amino acids based on their physico-chemical properties - we use H_n for polar (RKEDQN), neutral (GASTPHY) and hydrophobic (CVLIMFW) (Dubchak, Muchnik et al. 1999); V_n for small (GASCTPD), medium (NVEQIL) and large (MHKFRYW) (Dubchak, Muchnik et al. 1999); Z_n for of low polarizability (GASDT)(Dubchak, Muchnik et al. 1999), medium (CPNVEQI) and high (KMHFRYW) (Dubchak, Muchnik et al. 1999); P_n for low polarity (LIFWCMVY), neutral (PATGS) and high polarity (HQRKNED) (Dubchak, Muchnik et al. 1999); F_n for acidic (DE), basic (HKR), polar (CGNQSTY) and nonpolar (AFILMPVW); S_n for acidic (DE), basic (HKR), aromatic (FWY), small hydroxyl (ST), sulfur-containing (CM) and aliphatic (AGPILV); E_n for acidic (DE), basic (HKR), aromatic (FWY), small hydroxyl (ST), sulfur-containing (CM), aliphatic 1 (AGP) and aliphatic 2 (ILV). For clarity, these coding schemes are summarized in Table 6.

Support vector machines

Support vector machines (SVMs) are a set of related supervised learning methods used for classification and regression. Their common factor is the use of a technique known as the "kernel trick" to apply linear classification techniques to non-linear classification problems.

Suppose there are some data points which need to be classified into two classes. Often we are interested in classifying data as part of a machine-learning process. These data points can be multidimensional points. We are interested in whether we can separate them by a hyperplane (a generalization of a plane in three dimensional space to more than three dimensions). As we examine a hyperplane, this form of classification is known as linear classification. We also want to choose a hyperplane that separates the data points "neatly", with maximum distance to the closest data point from both classes -- this distance is called the margin. We desire this property since if we add another data point to the points we already have; we can more accurately classify the new point since the separation between the two classes is greater. Now, if such a hyperplane exists, the hyperplane is clearly of interest and is known as the maximum-margin hyperplane or the optimal hyperplane, as are the vectors that are closest to this hyperplane, which are called the support vectors.

We consider data points of the form: $\{(\mathbf{x}_1, c_1), (\mathbf{x}_2, c_2), \ldots, (\mathbf{x}_n, c_n)\}$ where the c_i is either 1 or −1 -- this constant denotes the class to which the point x_i belongs. We can view this as training data, which denotes the correct classification which we would like the SVM to eventually distinguish, by means of the dividing hyperplane, which takes the form

$$\mathbf{w} \cdot \mathbf{x} - b = 0.$$

As we are interested in the maximum margin, we are interested in the support vectors and the parallel hyperplanes (to the optimal hyperplane) closest to these support vectors in either class. It can be shown that these parallel hyperplanes can be described by equations

$$\mathbf{w} \cdot \mathbf{x} - b = 1, \qquad (1)$$

$$\mathbf{w} \cdot \mathbf{x} - b = -1. \qquad (2)$$

We would like these hyperplanes to maximize the distance from the dividing hyperplane and to have no data points between them. By using geometry, we find the distance between the hyperplanes being 2/|w|, so we want to minimize |w|. To exclude data points, we need to ensure that for all i either

$$\mathbf{w} \cdot \mathbf{x_i} - b \geq 1 \quad \text{or}$$

$$\mathbf{w} \cdot \mathbf{x_i} - b \leq -1$$

This can be rewritten as:

$$c_i(\mathbf{w} \cdot \mathbf{x_i} - b) \geq 1 \quad 1 \leq i \leq n. \qquad (3)$$

The problem now is to minimize |w| subject to the constraint (3). This is a quadratic programming (QP) optimization problem.

After the SVM has been trained, it can be used to classify unseen 'test' data. This is achieved using the following decision rule;

$$\hat{c} = \begin{cases} 1, & \text{if } \mathbf{w} \cdot \mathbf{x} + b \geq 0 \\ -1, & \text{if } \mathbf{w} \cdot \mathbf{x} + b \leq 0 \end{cases}$$

Writing the classification rule in its dual form reveals that classification is only a function of the Support vectors, i.e. the training data that lie on the margin. Here is the

picture in Figure 8 to describe the operation of support vector machines.

2-level support vector machine (SVM) model

The first level SVM classifiers comprise a number of separate SVM classifiers, each based on a specific sequence coding as described in the previous section. For the sake of notation simplicity, we will use the coding symbol to represent the SVM classifier based on that coding. For example, we will denote the SVM system comprising 3 classifiers, say, A, B and C by the shorthand symbol $A+B+C$. In this work, the first level classifiers consist of the following SVMs:

$\sum_{k=1}^{9} X_k^{a_1} + \sum_{k=0}^{6} D_k + \sum_{x \in S} X_5^x + \sum_{l \in S'} W_l$, where $S = \{H_3, P_3, F_3, S_2, E_2\}$ and $S' = \{7,\ldots,15\}$. Each SVM will generate a probability distribution(Yu, Wang et al. 2003; Yu, Lin et al. 2004) of the subcellular localization based on its particular sequence coding. A second SVM (i.e. the jury SVM) is used to process these probability distributions to generate the final probability distribution and the location with the largest probability is used as the prediction. The two-level SVM system is shown schematically in Figure 9.

Huffman tree model

Because of the unbalanced dataset which would lead to high accuracy but all the predictions focusing on just one classification, a Huffman tree model is constructed. Huffman coding is a method of lossless data compression, and a form of entropy encoding. The basic idea is to map an alphabet to a representation for that alphabet, composed of strings of variable size, so that symbols that have a higher probability of occurring have a smaller representation than those that occur less often. The Huffman's algorithm, key to Huffman coding, constructs an extended binary tree (Huffman tree) of minimum weighted path length from a list of weights.

A Huffman tree is a binary tree which minimizes the sum of f(*i*)D(*i*) over all leaves *i*, where f(*i*) is the frequency or weight of leaf *i* and D(*i*) is the length of the path from the root to leaf *i*. In each of the applications, f(*i*) has a different physical meaning. Here is an example of Huffman tree in Figure 10. It has the following properties: every internal node has 2 children; smaller frequencies are further away from the root; the 2 smallest frequencies are siblings.

In this work, every leaf on the Huffman tree means the classification of enzymes; the frequencies of each leaf mean the number of its enzyme class. According to previous statement, we have six nodes (leaves) at beginning and each of them has its own frequency.

The Huffman tree structure contains nodes, each of which contains a character, its frequency, a pointer to a parent node, and pointers to the left and right child nodes.The tree can contain entries for all 6 possible leaf nodes and all 5 possible parent nodes. At first there are no parent nodes. The tree grows by making successive passes through the existing nodes. Each pass searches for two nodes that have not grown a parent node and that have the two lowest weights. When the algorithm finds those two nodes, it allocates a new node, assigns it as the parent of the two nodes, and gives the new node a frequency count that is the sum of the two child nodes. The next iteration ignores those two child nodes but includes the new parent node. The passes continue until only one node with no parent remains. That node will be the root node of the tree. We can see the contribution of the Huffman tree step by step in Figure 11.

The Huffman tree model (see Figure 12) in this work is based on the Huffman tree previously constructed. Every node in this Huffman tree has a corresponding file. At a terminal node, the file is made including all data of the enzyme classifications; on the other hand, at an internal node, the file is made merging 2 files from the children of

this node. Besides, each internal node has an additional module. These corresponding modules include a SVM training set to help predict the enzyme classification.

Every input data should be put into the Huffman tree model from the tree root. By using 2-classification support vector machines, a decision must be made to predict which child the input data belongs to. Then this child node would be taken as a new tree root, and the steps above would be repeated until the new root has no child anymore. In the end the enzyme classification of the node the input data finally belongs to means the enzyme classification we predict.

RESULTS

Since there are many coding schemes in the protein structure, which coding schemes can be chosen to predict enzyme classification is important.

First, we calculate the accuracies of different coding schemes using multi-class SVMs and get the highest performance of 37.55% (shown in Table 7). Although the accuracy is higher than the one from Dobson and Doig, we can see the difference between these two: all predictions using multi-class SVMs are biased to EC 1, 2, 3, which class size are largest 3 in six enzyme classes (shown in Figure 13).

Second, we compare with the accuracies of the same method with recent research (Dobson and Doig 2005) but different coding schemes in order to make sure if such coding scheme can be used to predict enzyme class. The results are shown in Table 8. They all have similar accuracies with the range from 33% to 39%. Here is also accuracies comparison with different coding schemes in each enzyme class shown in Figure 14.

Then, we have to decide which coding schemes are used at each set in the Huffman tree model. Results for the best set accuracies in Huffman tree model with

each of these coding schemes are listed in Table 9. Since pursuing high accuracies in the model is not good enough for this predicting work, we take the best set MCC to generate this model. Results for the best set MCC in Huffman tree model with each of these coding schemes are listed in Table 10.

We generate Huffman tree model with different coding schemes at different set, such as C coding in set 7, D in set 8, X2 in set 9, H3X5 in set A, and finally N15C in set B. We pick up coding schemes in every set, some according to set MCC and some according to set accuracy in order to make a set of combinations to get the better accuracies in final model. All of combinations and the results for these combinations are listed at Table 11. Here is a comparison between the 2-level SVM method (Dobson and Doig 2005) and the Huffman tree method in this work in Figure 15.

DISCUSSION

Here are accuracies of different methods listed in Table 12 and Figure 16. We can use only multi-class SVMs to get best prediction of almost 40% accuracy, but the fact is that using multi-class SVMs to predict enzyme class cause the biased prediction. That is why we have to find the other way to do the un-biased prediction. We then use 2-level SVM model in order to get high accuracy and un-biased prediction at the same time. Using this model can have a 35% accuracy and a un-biased prediction (Dobson and Doig 2005) but it seems not enough.

There are some advantages for using Huffman tree model of predicting enzyme classification. Support vector machines are used for 2-classification distinguishing problem at first; all of other SVMs (such as multi-class SVMs) were created based on 2-class SVMs which are the simplest and effective classifiers. Predictions by using 2-classification SVMs seem reasonable in this work. Since the Huffman tree can decide which child the data belong to from two children at a time, the Huffman tree

model is most suitable using 2- classification SVMs.

The dataset in this work is unbalanced with the size of each EC number. Hydrolases, the largest size in this dataset, have 160 out of 498 enzymes (almost one-third) and ligases, the smallest size in this dataset, have only 20 which in size is one-eighth time than hydrolases. The prediction by using multi-class support vector machine would cause the extremely biased prediction, which means all of predicting data may lead to the same enzyme classification, hydrolases, and still get the prediction accuracy of 33%. Here are the results using multi-class SVMs in Table 7. It can get a 37% above accuracy by guessing only hydrolases and other enzymes which have more members in the dataset like oxidoreductases.

To avoid this biased prediction, Huffman tree model is used to solve the problem with unbalanced size of each classification. Every time we pick two nodes with smallest frequencies and create a new node gathered by these two nodes, we can have the assumption following: these two nodes have similarly small frequencies. The Huffman tree model can balance the size of two children of an internal node.

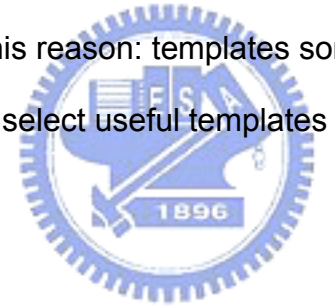
Two methods are described. One is 2-level SVM (Dobson and Doig 2005) method, which would at beginning separate one multi-classification problem into 15 one-class versus one-class sub-problems, then generate the prediction of the 15 sub-problems by using a one-versus-rest support vector classification approach. Another method is Huffman tree model. We successfully reduce 15 sub-problems to a five-set model. In other words, we can get similar accuracies during only five sub-problems in this work and it is important because the reduction of the number of sub-problems may cause the reduction of the computational time and space.

There are still some advices to get the improvement about the Huffman tree model. In Table 9 and Table 10, we can get neither high set accuracy nor set MCC. Although we can not have better prediction in oxidoreductases (EC 1) and

transferases (EC 2), here are the better predictions in hydrolases (EC 3), lyases (EC 4), and isomerases (EC 5). It tells us as long as we improve the accuracies of first layer in Huffman tree model we get higher total accuracy.

In addition, we can contribute other tree models in order to get better performance.

However, there are still something interesting discovering. In my coding schemes, both sequence-based and structure-based protein information are used. From Table 9 to Table 10, we can find out that using CSA templates (based on structural information) can not get better performance in our Huffman tree model. In common sense, structural information is considered more powerful since the same catalytic sites in proteins have more probabilities to be classified to the same enzyme class. This discovery can due to this reason: templates some of which may be useless are used, and it is necessary to select useful templates to get higher performance.



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TABLES

Table 1. The numbers of members and subclass in each enzyme

No.	*Class*	*Subclass number*	*Current membesr*
1	Oxidoreductase	22	1286
2	Transferase	9	1245
3	Hydrolase	13	1385
4	Lyase	7	422
5	Isomerase	6	167
6	Ligase	6	140

Table 2. Matrix of sequence identity (colored black) and Z-score (colored red) calculated between each other

	1bt1	*2nac*	*1apx*	*1ldm*	*1mbb*	*1gal*
1bt1	7.9	4.2	8.3	3.8	6.9	7.5
2nac	1.6	8.1	6.2	7.3	5.6	4.2
1apx	3.3	1.6	7.6	15.3	5.6	6.9
1ldm	2.0	4.4	1.6	8.0	8.8	2.8
1mbb	2.0	1.2	2.6	3.1	8.0	2.5
1gal	2.6	2.3	2.0	2.0	2.3	8.5

Table 3. Reactions of each protein, where the reaction occurs drown in red cycle

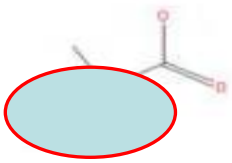
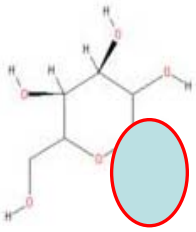
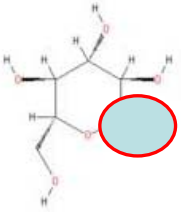
PDB ID *(EC No.)*	*Reaction mechanisms*
1ldm (1.1.1.27)	  (*S*)-lactate + NAD^+ = pyruvate + NADH + H^+
1mbb (1.1.1.158)	  UDP-N-acetylmuramate + $NADP^+$ = NADPH + H^++ UDP-N-acetyl-3-O-(1-carboxyvinyl)-D-glucosamine
1gal (1.1.3.4)	  -D-glucose + O_2 = D-glucono-1,5-lactone + H_2O_2

Table 4. The size of each enzyme group in the dataset

Enzyme Group (EC No.)	*Group size*
Oxidoreductases (1)	79
Transferases (2)	128
Hydrolases (3)	160
Lyases (4)	60
Isomerases (5)	51
Ligases (6)	20

Table 5.

Symbol	Properties	Dim
C	Protein composition of 20 amino acids	20
D	Protein composition of 20 amino acids by 2 continuous amino acids	400
Dj_n	Protein composition of 20 amino acids by 2 amino acids between which there are n other amino acids	400
N_nC	the amino acid composition of a sliding window of length centered on a given amino acid type. This provides information of the flanking sequences of a given amino acid type	400
X_n	Protein composition of 20 amino acids of n divisions	n* 20

Table 6.

Symbol (U_nX_5)	Properties	Regrouping member	Dim
H_3X_5	Hydrophobicity		135
	polar	RKEDQN	
	neutral	GASTPHY	
	hydrophobic	CVLIMFW	
V_3X_5	Normalized van der Waals volume		135
	0.00-2.78	GASCTPD	
	2.95-4.00	NVEQIL	
	4.43-8.08	MHKFRYW	
Z_3X_5	Polarizability		135
	0.000-0.018	GASDT	
	0.128-0.186	CPNVEQIL	
	0.219-0.409	KMHFRYW	
P_3X_5	Polarity		135
	4.9-6.2	LIFWCMVY	
	8.0-9.0	PATGS	
	10.4-13.0	HQRKNED	
F_3X_5	4 groups :		320
	acid	DE	
	base	HKR	
	polar	CGNQSTY	
	nonpolar	AFILMPVW	
S_2X_5	7 groups :		245
	acid	DE	
	base	HKR	
	amide	NQ	
	aromatic	FWY	
	Small hydroxyl	ST	
	sulfur	CM	
	aliphatic	AILVGP	
E_2X_5	8 groups :		320
	acid	DE	
	base	HKR	
	amide	NQ	

aromatic	FWY
small hydroxyl	ST
sulfur	CM
aliphatic 1	AGP
aliphatic 2	ILV

Table 7. accuracy comparison with different coding using multi-class SVMs.

Protein info.	*Accuracy (%)*
C	34.94
D	34.94
Dj_1	34.94
Dj_2	34.94
Dj_3	33.33
Dj_4	34.54
Dj_5	34.74
Dj_6	35.94
N_3C	34.94
N_5C	35.94
N_7C	32.73
N_9C	35.94
$N_{11}C$	36.55
$N_{13}C$	35.74
$N_{15}C$	35.74
E_2X_5	33.94
F_3X_5	34.14
H_3X_5	34.94
P_3X_5	33.94
S_2X_5	33.53
V_3X_5	33.94
Z_3X_5	33.33
X_2	36.14
X_3	37.55
X_4	36.95
X_5	34.14
X_6	33. 33
X_7	33.13
X_8	35.74
X_9	32.73
CSA	33.60

Table 8. Rank accuracy comparison with the same method but different protein information

	Cumulative accuracy (%) by rank					
Protein info.	1	2	3	4	5	6
Doig	34.94	60.00	77.00	86.00	96.00	100.00
C	39.56	49.84	76.10	89.16	95.98	100.00
D	34.14	60.04	74.50	86.95	95.98	100.00
Dj_1	34.34	59.04	74.30	86.95	95.78	100.00
Dj_2	33.33	59.44	74.70	87.15	95.98	100.00
Dj_3	32.93	58.63	73.29	85.94	95.98	100.00
Dj_4	35.54	61.65	75.70	86.35	95.98	100.00
Dj_5	32.73	58.23	73.49	83.73	95.78	100.00
Dj_6	34.34	59.04	75.70	86.35	95.98	100.00
N_3C	31.53	59.44	73.90	85.34	95.98	100.00
N_5C	33.73	61.45	75.30	88.15	95.98	100.00
N_7C	38.35	64.86	79.32	88.15	96.59	100.0
N_9C	34.14	59.44	74.90	85.94	95.78	100.00
$N_{11}C$	*34.14*	*59.24*	*76.91*	*86.55*	*95.98*	*100.00*
$N_{13}C$	33.94	59.44	74.94	85.34	95.98	100.00
$N_{15}C$	35.34	61.24	76.91	85.94	95.58	100.00
E_2X_5	32.73	57.63	73.69	83.94	95.78	100.00
F_3X_5	33.53	58.03	74.30	87.15	95.98	100.00
H_3X_5	38.35	61.85	76.10	85.94	95.78	100.00
P_3X_5	32.53	58.63	74.30	85.14	95.98	100.00
S_2X_5	34.14	59.04	74.30	85.34	95.98	100.00
V_3X_5	36.35	61.24	73.69	84.94	95.98	100.00
Z_3X_5	34.94	62.65	77.31	87.55	95.98	100.00
X_2	36.95	61.04	74.10	84.94	95.98	100.00
X_3	33.53	62.05	79.12	86.75	96.18	100.00
X_4	36.75	61.04	76.31	88.76	95.78	100.00
X_5	36.35	60.44	75.90	84.94	95.78	100.00
X_6	35.54	60.04	76.10	88.15	95.98	100.00
X_7	36.14	63.45	77.71	89.36	95.58	100.00
X_8	35.34	61.65	77.31	87.75	95.98	100.00
X_9	31.12	60.24	75.10	85.34	95.98	100.00
CSA	30.32	43.17	61.85	71.89	84.54	100.00

Table 9. Set accuracy (%) of each protein information

Protein info.	*set7*	*set8*	*set9*	*setA*	*setB*
C	71.8	61.8	72.5	64.3	59.4
D	73.2	59.5	66.2	65.3	58.6
Dj_1	73.2	55.7	69.6	65.6	58.8
Dj_2	73.2	61.8	69.1	63.2	59.8
Dj_3	73.2	58.0	66.7	63.9	59.4
Dj_4	73.2	59.5	65.7	67.4	58.6
Dj_5	71.8	55.0	71.0	67.4	59.2
Dj_6	73.2	57.3	65.7	64.6	60.4
N_3C	73.2	54.2	65.7	65.3	59.0
N_5C	71.8	58.0	68.1	66.7	59.0
N_7C	71.8	59.5	70.0	65.3	58.6
N_9C	71.8	57.3	71.0	66.3	59.2
$N_{11}C$	71.8	61.1	68.1	68.7	60.0
$N_{13}C$	71.8	67.2	69.1	67.4	60.8
$N_{15}C$	71.8	61.8	69.1	69.4	60.8
E_2X_5	73.2	55.7	68.6	64.9	59.0
F_3X_5	71.8	55.0	63.3	67.4	58.8
H_3X_5	74.6	59.5	68.1	63.6	58.8
P_3X_5	73.2	60.3	62.3	64.3	59.0
S_2X_5	74.6	56.5	66.2	65.6	59.4
V_3X_5	73.2	55.7	69.6	62.9	59.6
Z_3X_5	73.2	55.0	67.1	64.6	60.0
X_2	71.8	63.4	72.0	66.7	58.4
X_3	73.2	58.8	73.4	63.6	59.2
X_4	73.2	56.5	74.4	66.3	58.6
X_5	73.2	58.0	72.9	66.0	59.0
X_6	73.2	58.8	72.0	65.3	58.8
X_7	73.2	57.3	69.6	65.3	58.6
X_8	71.8	55.7	72.0	64.9	58.8
X_9	73.2	60.0	72.5	65.3	59.6
CSA	73.2	62.3	67.1	59.8	59.4

Table 10. Set MCC (%) of each protein information

Protein info.	*set7*	*set8*	*set9*	*setA*	*setB*
C	27.2	19.1	35.8	22.0	13.3
D	19.1	18.8	31.6	27.9	10.8
Dj_1	27.2	19.0	33.4	29.7	10.1
Dj_2	27.2	18.7	28.2	23.6	11.5
Dj_3	19.1	15.2	21.4	23.1	11.0
Dj_4	27.2	23.5	24.8	23.6	14.1
Dj_5	27.2	18.1	28.3	28.0	12.1
Dj_6	19.1	15,7	30.0	30.8	14.4
N_3C	14.7	14.8	28.2	25.6	11.3
N_5C	18.0	12.1	34.6	27.1	10.8
N_7C	14.4	19.3	39.3	24.1	8.8
N_9C	15.3	28.0	37.2	25.4	13.3
$N_{11}C$	13.2	24.1	37.0	25.3	14.9
$N_{13}C$	19.1	34.7	35.9	24.0	11.8
$N_{15}C$	21.4	30.4	37.0	25.1	17.9
E_2X_5	19.1	26.2	38.2	25.3	9.6
F_3X_5	19.0	18.0	15.8	24.5	10.7
H_3X_5	13.2	23.4	33.6	27.2	11.3
P_3X_5	20.7	17.4	16.5	23.1	10.8
S_2X_5	28.7	22.3	33.3	29.3	12.1
V_3X_5	19.1	21.9	30.6	28.8	11.2
Z_3X_5	30.5	24.1	27.7	22.1	10.7
X_2	19.1	23.0	43.6	28.0	11.9
X_3	25.4	17.7	37.9	27.6	13.8
X_4	13.2	24.3	45.2	23.1	15.0
X_5	13.2	17.2	36.5	23.9	11.9
X_6	19.1	15.1	34.6	21.2	12.1
X_7	13.2	18.0	31.9	27.4	14.3
X_8	19.1	27.3	47.2	25.6	11.9
X_9	21.4	16.7	34.9	27.6	11.0
CSA	10.7	25.7	34.6	19.5	12.4

Table 11. Accuracies using Huffman tree model

Coding scheme in different set					*Accuracy(%)*
Set 7	*Set 8*	*Set 9*	*Set A*	*Set B*	
Z3X5	N13C	X4	S2X5	Dj6	34.94
Z3X5	N15C	N9C	N11C	Dj6	36.14
Z3X5	N15C	X4	N11C	Dj6	36.14
Z3X5	N13C	X4	Dj6	Dj6	34.34
Z3X5	N13C	X4	Dj1	Dj6	34.34
Z3X5	N13C	X8	Dj1	Dj6	33.94
Z3X5	N15C	X2	N11C	N15C	35.54

Table 12. Accuracy comparison with different methods and different protein information

Method	*Coding schemes*	*Accuracy(%)*
2-level SVM	from Dobson & Doig	35.00
2-level SVM	C	39.56
SVM only	X3	37.55
Huffman tree model	Z3X5+ N15C+N9C+N11C+Dj6	36.14

FIGURE CAPTIONS

FIGURES

Figure 1. The number of proteins with unknown function in PDB

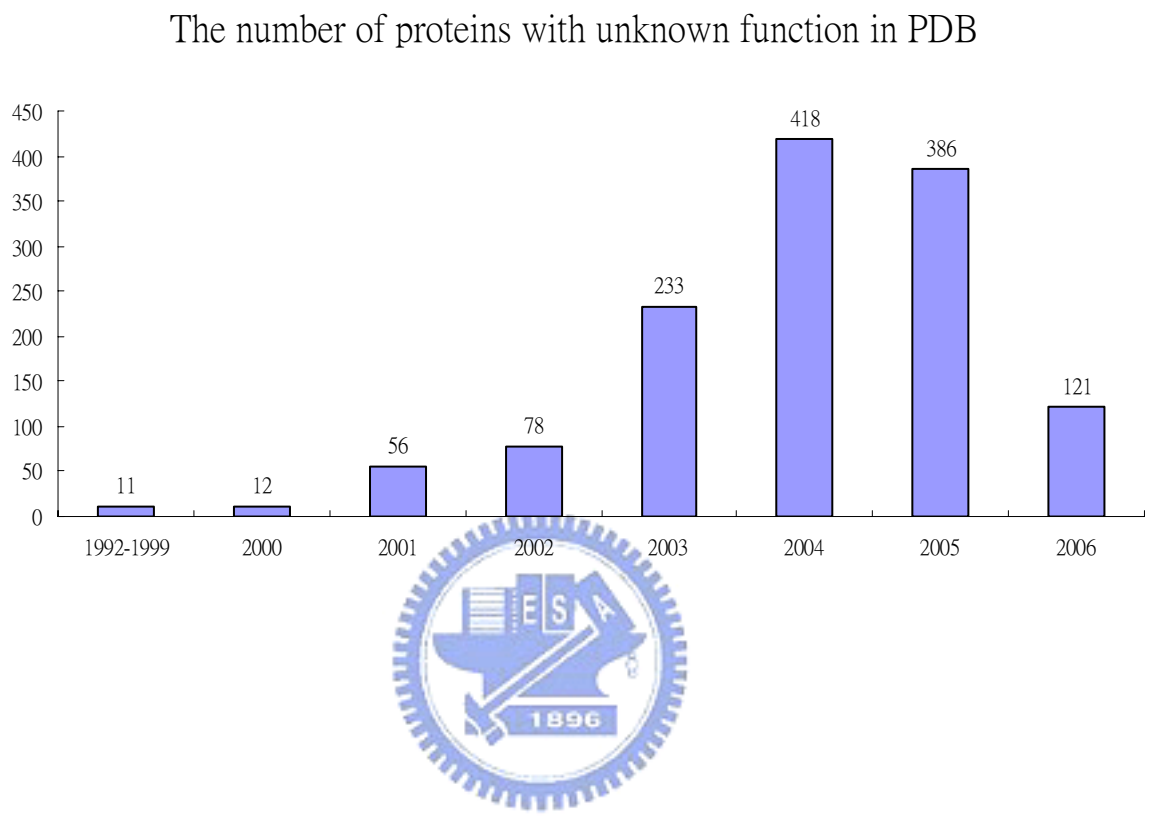
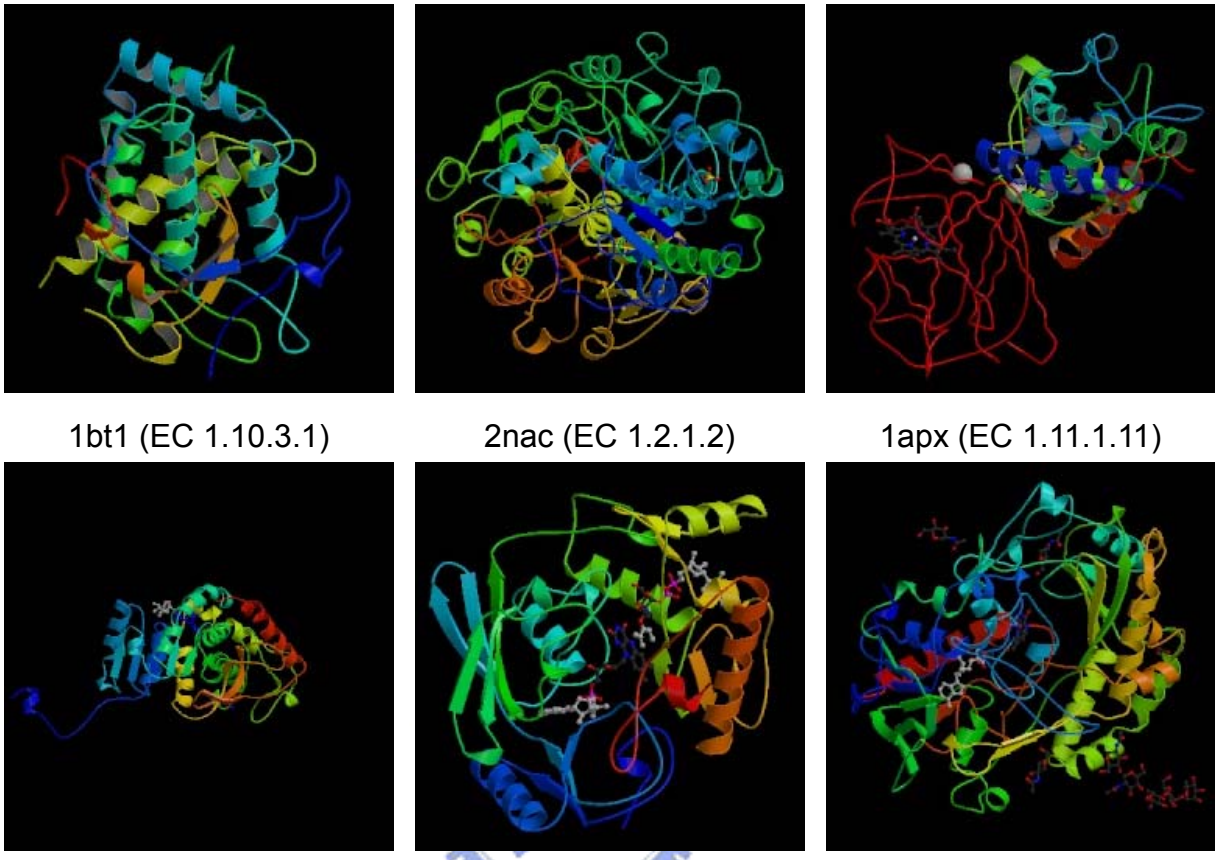


Figure 2.Examples of oxidoreductases

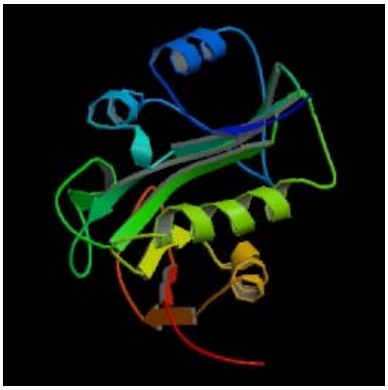


1bt1 (EC 1.10.3.1) 2nac (EC 1.2.1.2) 1apx (EC 1.11.1.11)

1ldm (EC 1.1.1.27) 1mbb (EC 1.1.1.158) 1gal (EC 1.1.3.4)

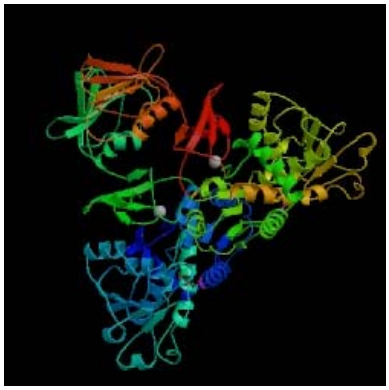
Figure 3. Examples of transferases

1bs0 (EC 2.3.1.47)

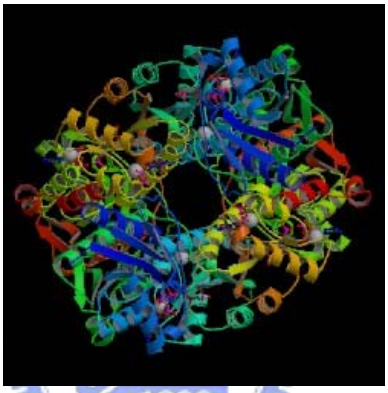


1b6b (EC 2.3.1.87)

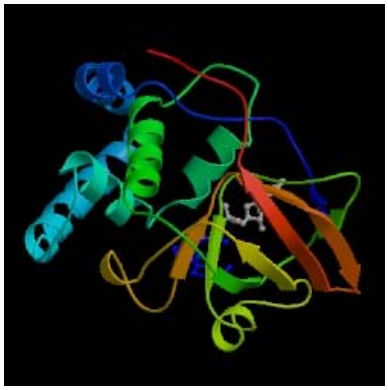
2tps (EC 2.5.1.3)



1at1 (EC 2.1.3.2)

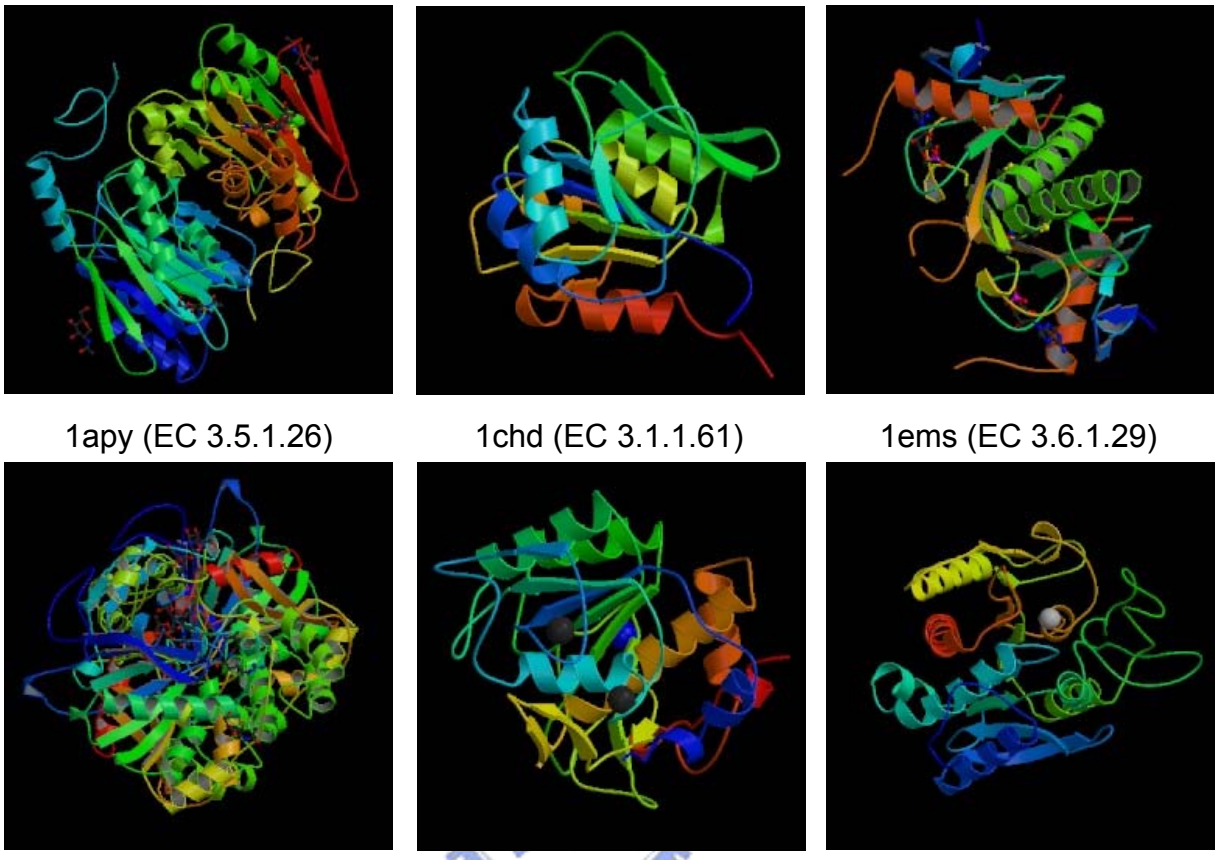


1pfk (EC 2.7.1.11)



1og1 (EC 2.4.2.31)

Figure 4. Example of hydrolases



1apy (EC 3.5.1.26) 1chd (EC 3.1.1.61) 1ems (EC 3.6.1.29)

1bcr (EC 3.4.16.6) 1sca (EC 3.4.21.6) 5cpa (EC 3.4.17.1)

Figure 5. Flow chart

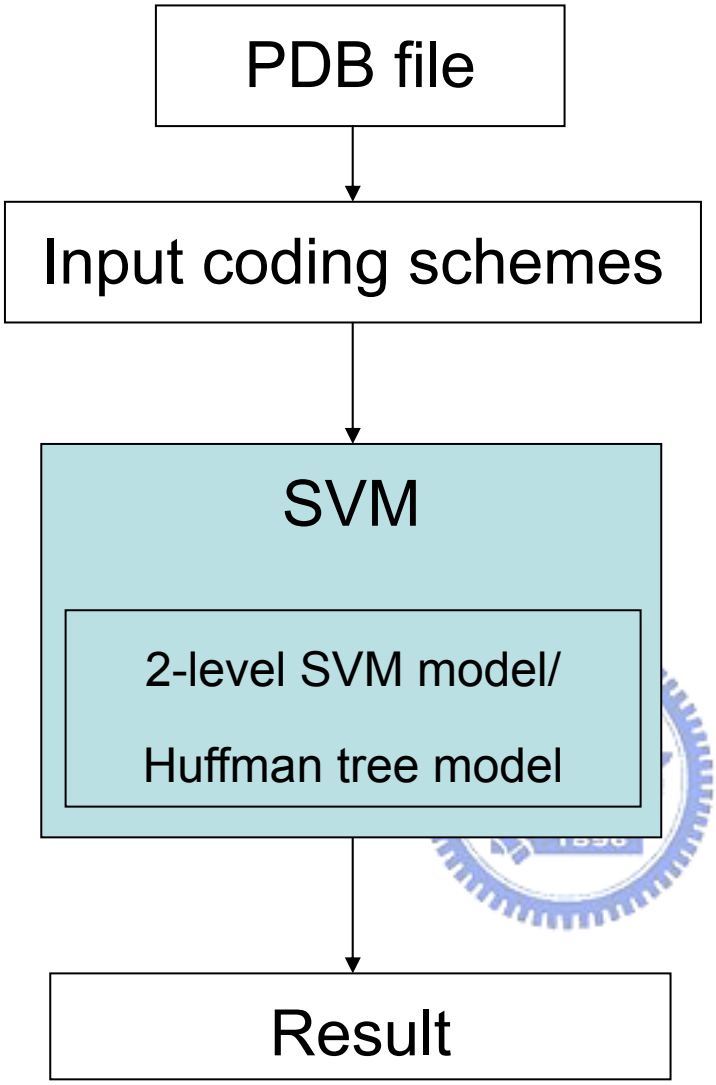
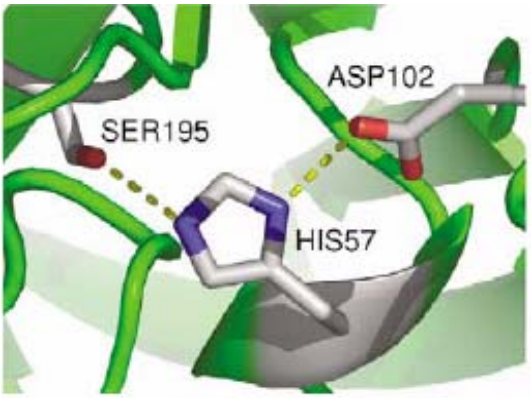


Figure 6. Example: CSA template of trypsin



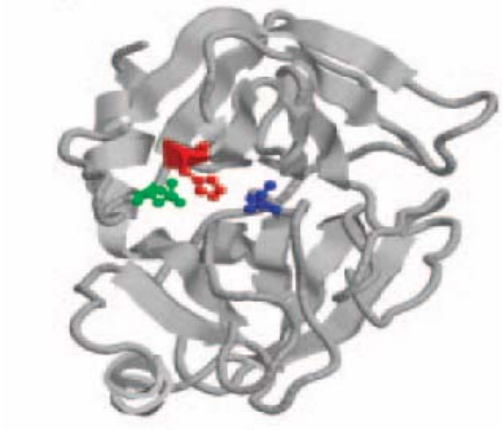


Figure 7. CSS example of the output

Catalytic site search results

Layout of these results

These results begin with a summary of all template matches to your query structure. This summary lists the CSA entry that the template was based on, and the quality of the match. Further down the page, the details of the residues involved in each template match are described. Clicking on the **Details** link for a match in the summary section will take you straight to the details for that match. Be aware that this server returns all matches, regardless of their quality. The **Assessment** column only gives a very rough guide to the accuracy of a match. You should look at both the E-value and RMSD of a match before investigating it further.

Summary

Hit rank	Template	Description in PDBsum	RMSD	E-value	Assessment	
1	1apy	Aspartylglucosaminidase	1.18	0.012	Possible	Details
2	1cjw	Serotonin n-acetyltransfe...	1.44	0.02	Possible	Details
3	2npx	Nadh peroxidase (e.c.1.11...	1.25	0.04	Possible	Details
4	3nse	Nitric oxide synthase	1.37	0.088	Possible	Details
5	1zrm	L-2-haloacid dehalogenase	1.03	0.099	Possible	Details
6	1s2i	Purine trans deoxyribosylase	1.13	0.27	Unlikely	Details

Full details

Hit rank 1

CSA entry for template	Template	RMSD	E-value	Assessment
1apy	Aspartylglucosaminidase	1.18	0.012	Possible

Residues in template 1apy			Matching residues in query structure 1fpb		
Residue name	Residue number	Chain	Residue name	Residue number	Chain
SER	49	A	SER	36	A
THR	183	B	SER	88	A
THR	201	B	THR	39	A
THR	234	B	ALA	40	A

Figure 8. Support vector machine

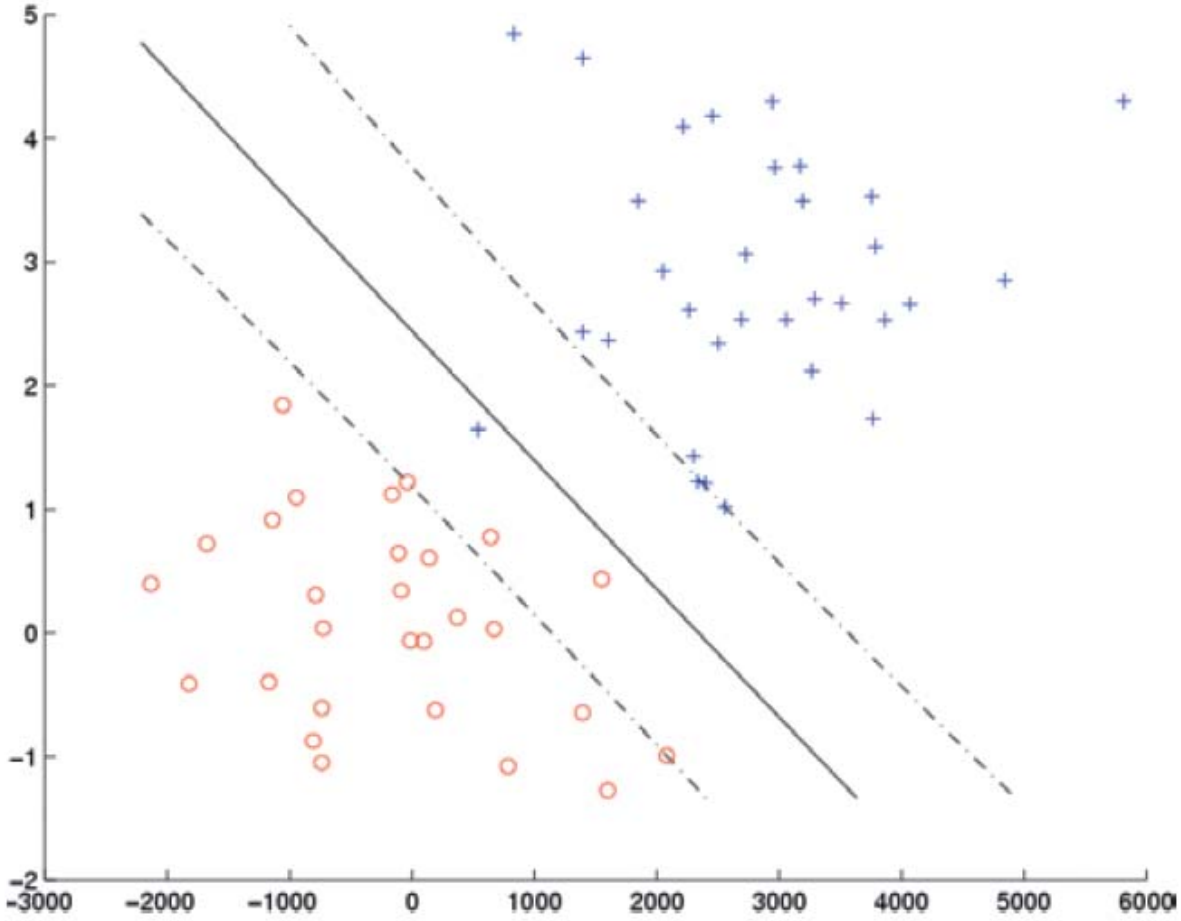


Figure 9. 2-level SVM

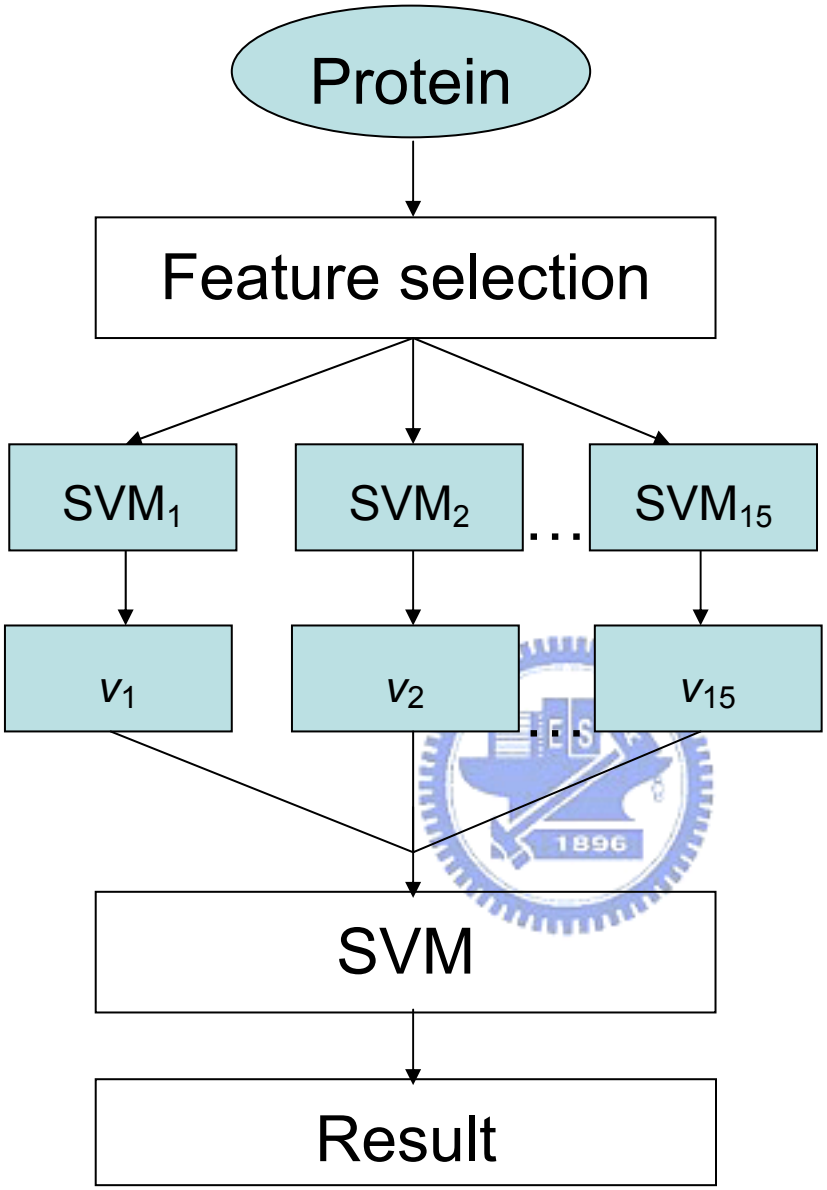


Figure 10. example of Huffman tree

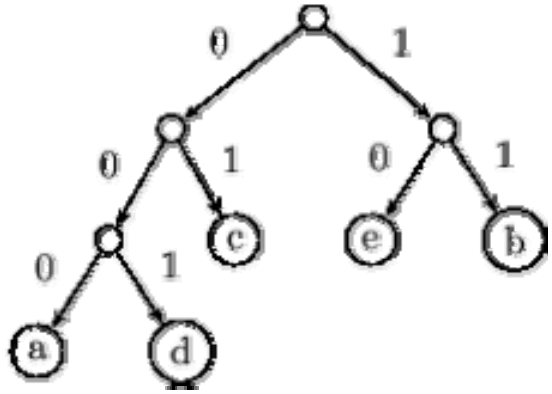
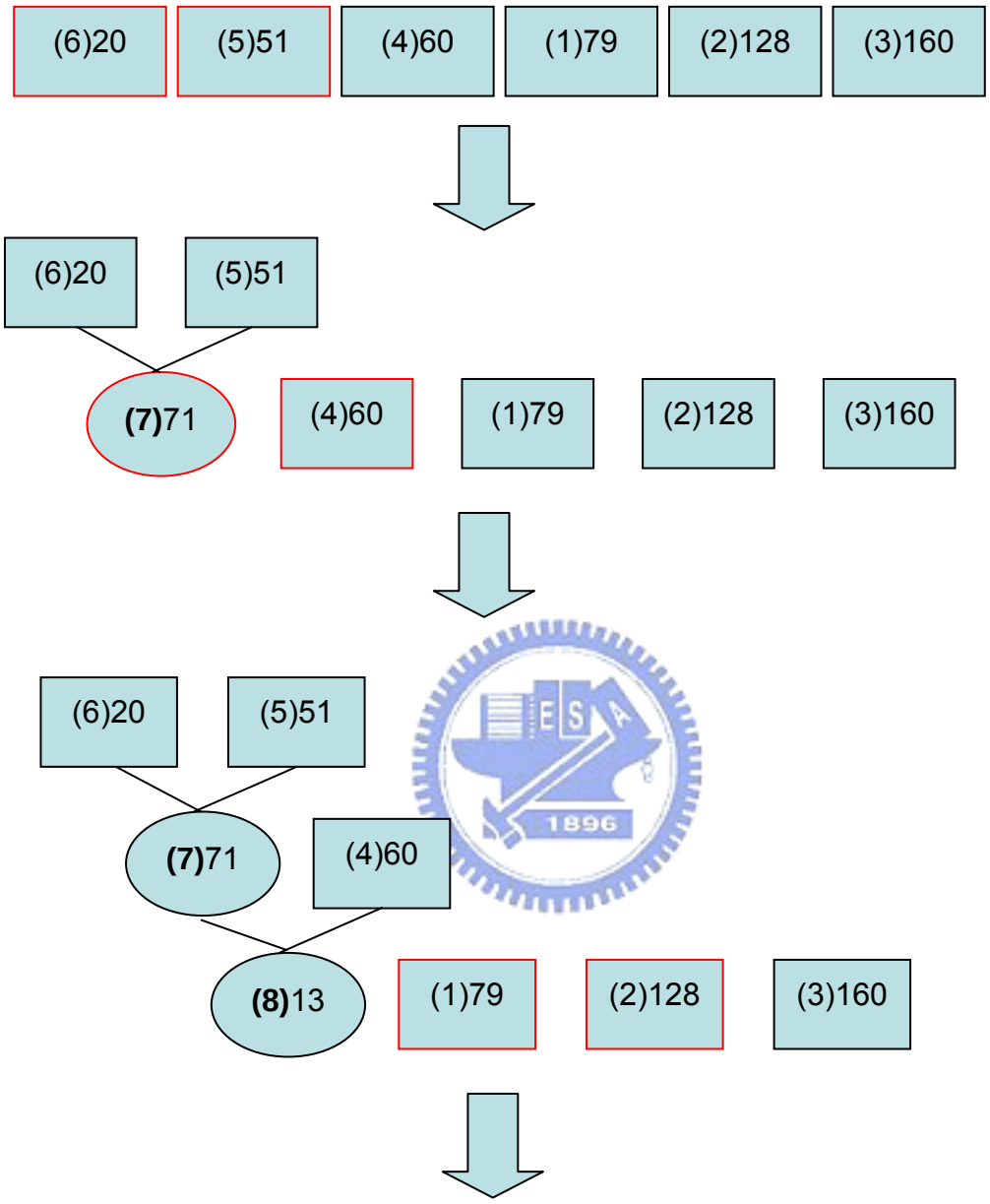


Figure 11. The construction of the Huffman tree step by step



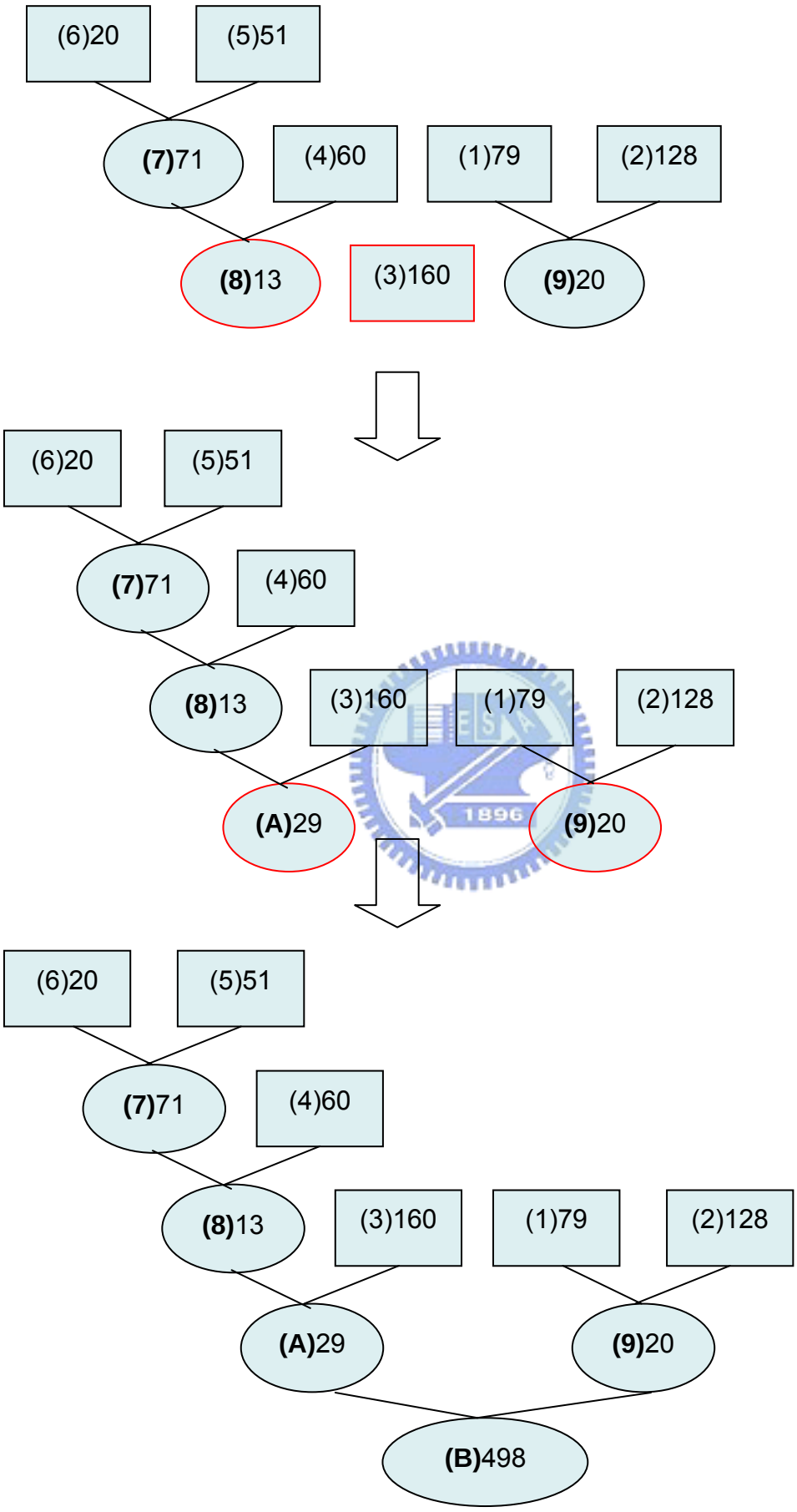

(6)20
(5)51
(7)71
(4)60
(1)79
(2)128
(8)13
(3)160
(9)20
(6)20
(5)51
(7)71
(4)60
(8)13
(3)160
(1)79
(2)128
(A)29
(9)20
(6)20
(5)51
(7)71
(4)60
(8)13
(3)160
(1)79
(2)128
(A)29
(9)20
(B)498

Figure 12. Huffman tree model in this work

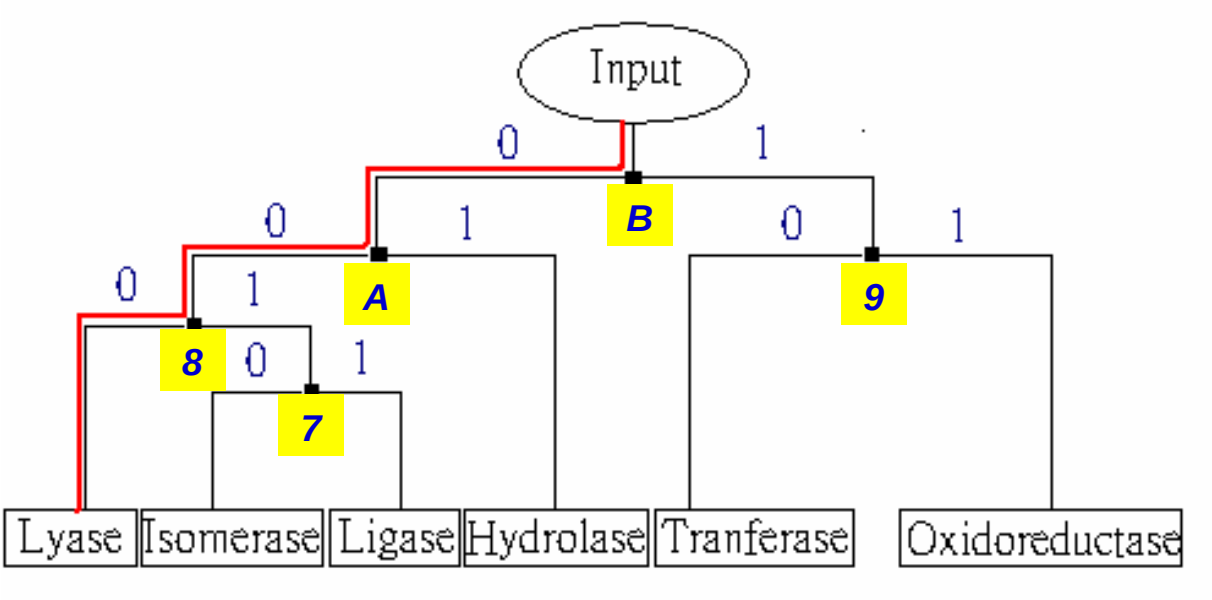


Figure 13. Class accuracies comparison with 2 different methods (multi-class SVM and 2-level SVM model)

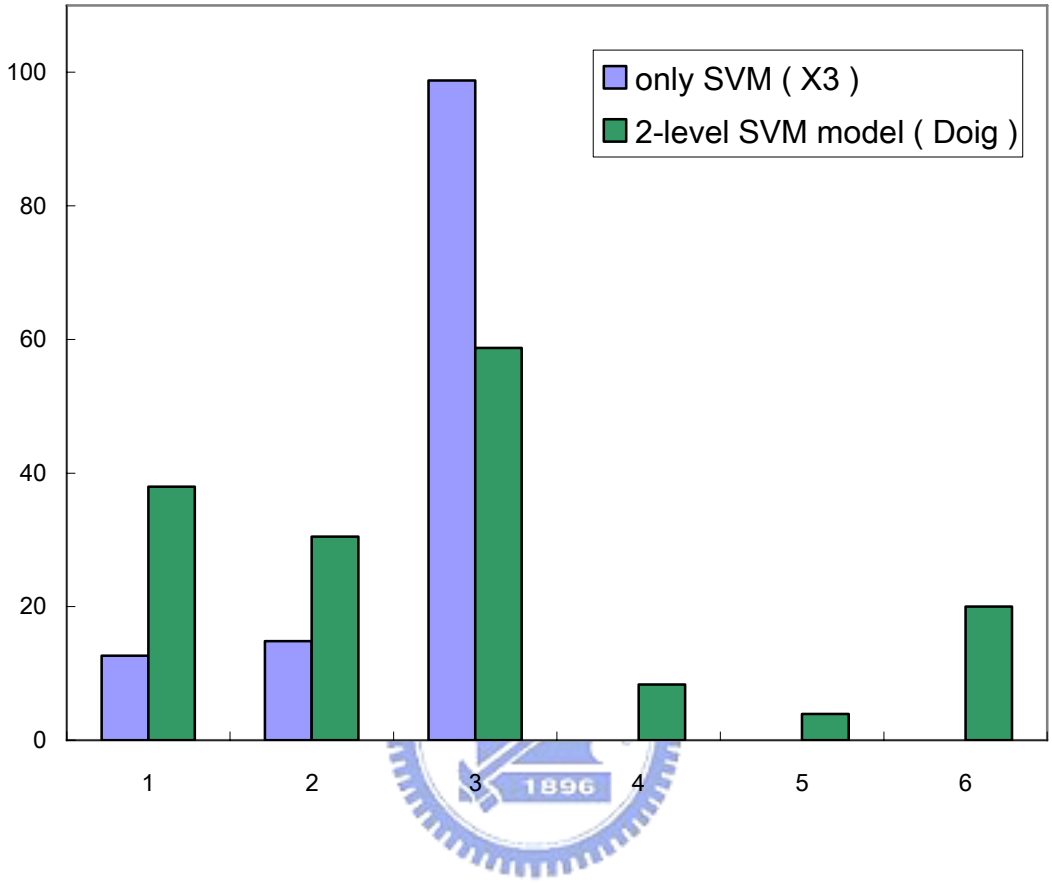


Figure 14. Class accuracies comparison with different coding schemes using 2-level SVM method

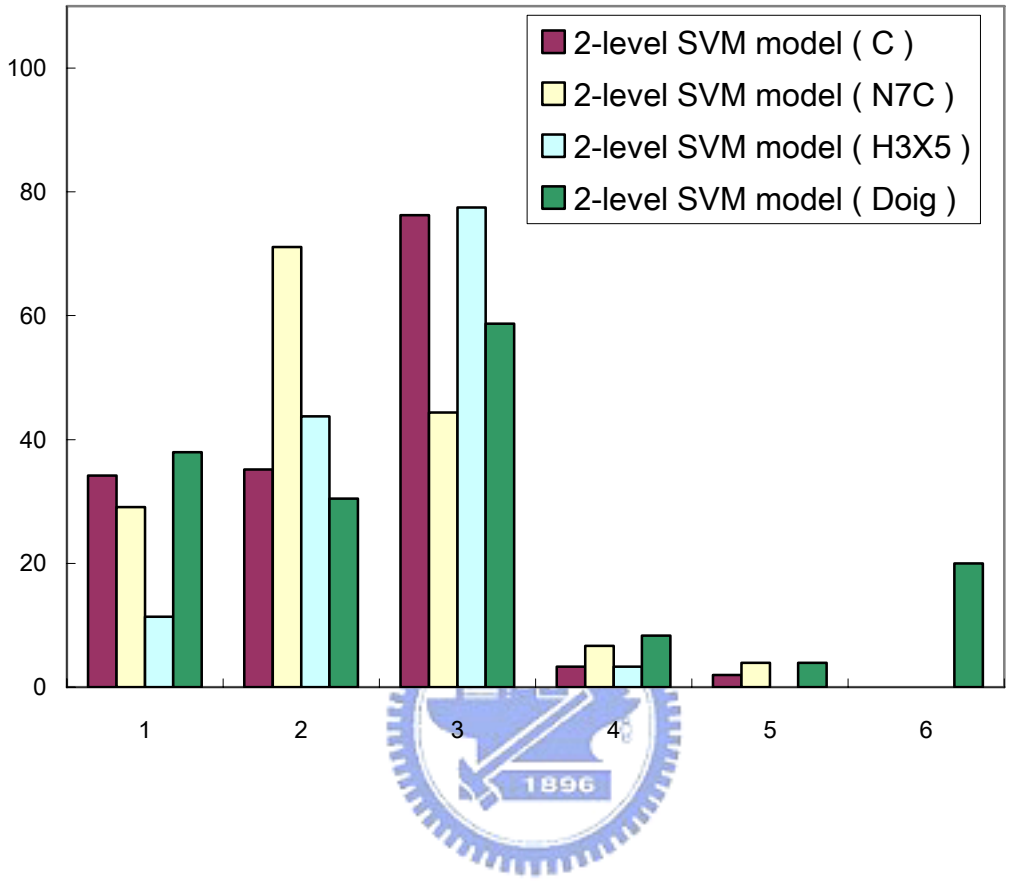


Figure 15. Class accuracies comparison with different methods (2-level SVM model and Huffman tree model)

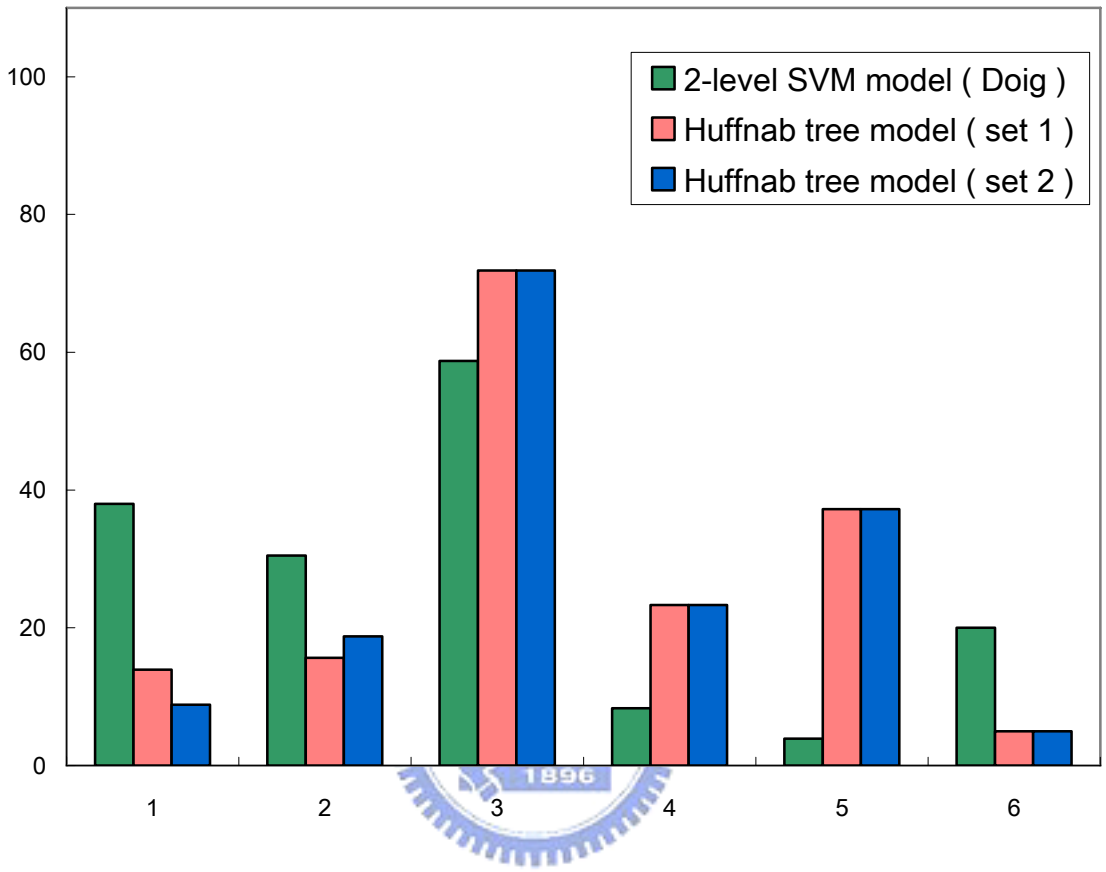
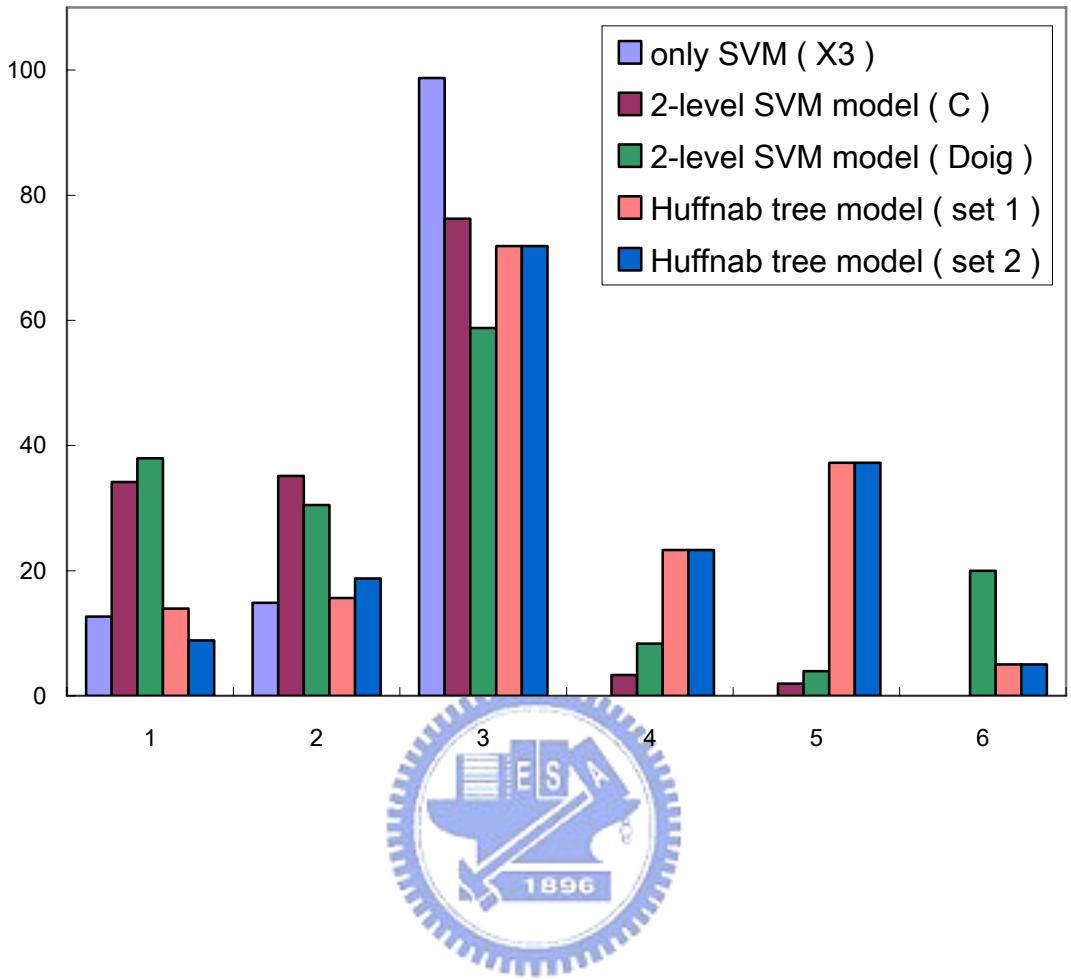


Figure 16. Class accuracies comparison with all methods



APPENDIX

Description of each enzyme classification

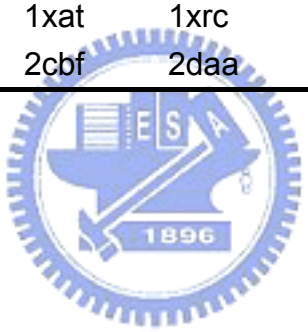
Group	*Reaction catalyzed*	*Typical reaction*	*Enzyme example(s) with trivial name*
EC 1 Oxidoreductases	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	AH + B → A + BH (reduced) A + O → AO (oxidized)	Dehydrogenase, oxidase
EC 2 Transferases	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phospate group	AB + C → A + BC	Transaminase, kinase
EC 3 Hydrolases	Formation of two products from a substrate by hydrolysis	AB + H2O → AOH + BH	Lipase, amylase, peptidase
EC 4 Lyases	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	RCOCOOH → RCOH + CO2	
EC 5 Isomerases	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	AB → BA	Isomerase, mutase
EC 6 Ligases	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP	X + Y+ ATP → XY + ADP + Pi	Synthetase

Dataset (Oxidoreductases / EC No. 1)

1a8q	1aor	1arx	1b4u	1b5t	1ba3	1bug	1ci0
1cp2	1cpo	1cpt	1cq1	1d4o	1d6u	1d7c	1do6
1dtw	1e1d	1eb7	1en6	1evi	1f0y	1ff3	1fp4
1geu	1goh	1gqh	1h2a	1h4i	1hb1	1hfe	1hlr
1hqt	1htp	1hwj	1i9d	1ik3	1ika	1ikt	1ivj
1jl3	1jpu	1knd	1l1d	1l5t	1l6p	1m41	1me8
1mhc	1mj4	1mro	1ndo	1ndt	1niw	1nox	1oya
1phm	1qav	1qbg	1qdb	1qg0	1qi1	1rx8	1sur
1sxz	1uox	1vao	1vif	1vnf	1xik	2aop	2bbk
2dmr	3mde	3pcm	4nos	5r1r	6pah	8cat	

Dataset (Transferases / EC No. 2)

1a59	1a6j	1b4f	1b7b	1bdf	1ble	1bmt	1bo1
1btk	1c2p	1c41	1c4g	1ckn	1cm1	1d0q	1dd9
1dy3	1dzf	1e0c	1e2a	1e2o	1e6v	1efz	1eh8
1ejc	1ep9	1ew0	1eye	1ez1	1ezf	1f0l	1f0n
1f75	1f7t	1f8y	1ffs	1fgg	1ftr	1g0w	1g2p
1g5h	1g6c	1g6g	1g71	1gjv	1gmi	1gms	1gno
1gpb	1gpu	1gq5	1gz0	1h17	1h54	1hav	1hkc
1hml	1hxq	1hzw	1i2n	1ig3	1iib	1ik7	1im8
1iu4	1ixm	1jg9	1jho	1jkx	1k04	1k1f	1k30
1k47	1k9s	1k9v	1kgy	1kgz	1khc	1ki8	1kq4
1kzh	1kzl	1l2q	1ld8	1lii	1liu	1lkl	1lp4
1lqp	1lt8	1m3k	1m4j	1m6b	1m9z	1mby	1ml9
1moq	1msk	1n06	1nh7	1nm2	1nom	1nun	1pdo
1poi	1ptq	1qap	1qd1	1qf8	1qjc	1qsm	1rgs
1shf	1vpe	1vsf	1xat	1xrc	1xtc	1zym	22gs
2a0b	2bef	2can	2cbf	2daa	2f3g	2jdx	2pol



Dataset (Hydrolases / EC No. 3)

1a17	1a2o	1a2t	1a6f	1a6q	1a79	1ak0	1alw
1amp	1aqt	1aug	1auo	1avg	1ayd	1az9	1b65
1b6m	1b79	1b9v	1bgo	1bir	1bpl	1bwr	1c77
1c8u	1ceb	1cel	1cfr	1cfz	1cjy	1cqy	1ctt
1cug	1cvm	1cwt	1d8i	1dix	1dnk	1dup	1dx5
1e1a	1e44	1e7d	1em9	1eni	1eoj	1ewn	1ex1
1f0j	1fce	1fcm	1fo2	1fo6	1fpb	1fw3	1fwg
1g0s	1g2i	1g9z	1gny	1gpp	1gtp	1gye	1gym
1h70	1h8g	1hja	1ho3	1hrt	1hv5	1hxk	1hzf
1i3j	1i3o	1i4o	1i74	1i78	1i8a	1icf	1ici
1ief	1ifs	1ihj	1iod	1iqb	1isj	1iw8	1j98
1j9l	1jh7	1jhc	1jke	1jt9	1jys	1k3b	1k46
1k5c	1k82	1kg7	1khl	1kie	1klx	1ko7	1kvc
1ky4	1l2p	1l7n	1lau	1lba	1lbu	1lkt	1lmh
1ln0	1m9n	1mc0	1mc9	1mml	1mt5	1mu7	1n64
1n8o	1nb3	1nba	1ngh	1nzy	1o0w	1pex	1png
1prx	1ptt	1pvc	1qaz	1qbj	1qcn	1qdn	1qfx
1qk2	1qlm	1qum	1rbd	1scn	1svb	1ush	1wgi
1xo1	2acy	2bqk	2dyn	2kai	2mjp	2phi	2pth
2reb	3bc2	3eng	3pva	3ygs	5pnt	5rla	6fit

Dataset (Lyases / EC No. 4)

1ahj	1ayl	1b66	1b6r	1ca2	1csh	1csm	1d7a
1dch	1dci	1dnp	1doz	1dp4	1dqs	1dwk	1dxe
1e51	1e9n	1ebm	1egh	1et0	1f3t	1fgh	1fi4
1fro	1fuo	1fx4	1gqo	1gxo	1i6o	1i7o	1iv1
1j58	1jbq	1jd3	1jl0	1jr2	1ju2	1juk	1k0e
1k8t	1k8w	1kep	1kiz	1kko	1lk9	1mka	1n7n
1n8w	1nbw	1o8f	1pda	1ppp	1qpb	1qrl	1rus
1uro	2fua	2yas	3std				

Dataset (Isomerases / EC No. 5)

1a41	1am2	1amu	1b73	1b9l	1bgw	1bkf	1bkh
1bwz	1com	1cy9	1d3y	1dea	1did	1e59	1eej
1ek6	1epz	1eqj	1eyq	1f2v	1f6d	1fp3	1fui
1g58	1hnu	1i8t	1i9a	1iv8	1j5s	1jc4	1jof
1k0w	1lvh	1lzo	1mx0	1nsu	1nuh	1ois	1pii
1pym	1qjg	1sqc	1vcc	1vkl	2req	2sfp	3gsb
4csm	4otc	5cyh					

Dataset (Ligases / EC No. 6)

1a48	1a8h	1ayz	1b04	1b47	1bdo	1cg1	1cli
1ct9	1d5f	1e4e	1eeh	1h4s	1htq	1i2t	1ik9
1mdb	1mkh	1qmh	1ycr				

Representative CSA templates for a non-overlapping set of 147 families

12as	1km1	1c1h	1hz1	1gal	3fit	1b8a	1kdn
1fmt	1oya	1ql0	1kkt	1avw	1wab	1d8w	1pgn
1qgy	1xid	1a2f	1dnw	1afr	1nux	1lam	3er3
1k9z	1ucc	1d8h	2nac	1l1n	1pnt	1lta	1cy8
1osj	1jf9	1hbz	1cjw	1cg0	1f3t	1raf	1gxz
1gpi	4pfk	2lip	2dnj	1kf4	1hmp	1hl2	1ryb
1foh	5csm	1apy	1knf	1m1t	1jep	1pzh	1bcr
1zrm	1nwc	1kta	2npx	1gpf	1pow	2ayh	1ei9
1ndz	6enl	1dd8	1edd	1ftw	5yas	1aur	1onr
1chd	1pfq	1naw	1owp	1h1y	1vgv	1egm	1djq
1nf5	3nse	1ghs	1ah3	1aj0	1pyd	1brl	1bd4
1bvw	1dj9	1dah	1b0p	1cwy	1n7n	1iov	1qnt
1k7x	1qtq	1e2h	1b73	1gpr	1jp3	2ay6	1opm
1cns	1pj5	3uag	1pj4	1yjb	1gpk	1diz	2tps
1g9r	1hdq	1os1	1um9	1csc	1gon	1mas	1jz7
1qpq	1nyy	1dcp	1h17	1nhc	1f61	1dty	1mbb
1gyc	1bg4	1fwc	1b8f	1h7p	1gqn	1uou	1f17
1n1p	1say	1a9s	1ecj	1sry	1hor	1djl	1qfm
1fdj	1s2i	1p7t