VOT 74288

THE DEVELOPMENT OF MACHINE LEARNING BASED SOFTWARE FOR PREDICTING PROTEIN-PROTEIN INTERACTIONS AND PROTEIN FUNCTION FROM PROTEIN PRIMARY STRUCTURE

(PEMBANGUNAN PERISIAN BERASASKAN PEMBELAJARAN MESIN UNTUK MERAMAL INTERAKSI PROTEIN-PROTEIN DAN FUNGSI PROTEIN DARIPADA STRUKTUR PRIMARI PROTEIN)

MUHAMAD RAZIB BIN OTHMAN
SAFAAI BIN DERIS
HANY TAHER AHMED ALASHWAL
ROSLI BIN MD. ILLIAS
SAFIE BIN MAT YATIM

RESEARCH VOT NO:
74288

Jabatan Kejuruteraan Perisian
Fakulti Sains Komputer Dan Sistem Maklumat
Univerisit Teknologi Malaysia

2007

ABSTRACT

Understanding proteins functions is a major goal in the post-genomic era. Proteins usually work in context of other proteins and rarely function alone. Therefore, it is highly relevant to study the interaction partners of a protein in order to understand its function. For this reason, the main objective of this thesis is to predict protein-protein interactions based only on protein primary structure. Using the Support Vector Machines (SVM), different protein features have been studied and examined. These features include protein domain structures, hydrophobicity and amino acid compositions. The results imply that the protein domain structure is the most informative feature for predicting protein-protein interactions. It also requires much lower running time compared to the other features. However, using normal binary SVM requires positive and negative data samples. Although it is easy to get a dataset of interacting proteins as positive examples, there are no experimentally confirmed non-interacting proteins to be considered as negative examples. Previous researches cope with this problem by artificially generate random set of proteins pairs that are not listed in the Database of Interacting Proteins (DIP) as negative examples. This approach can be used for comparing features because the error will be uniform. In this research, we consider this problem as a one-class classification problem and solve it using the One-Class SVM. Using only positive examples (interacting protein pairs) in training phase, the one-class SVM achieves accuracy of 80%. These results imply that protein-protein interaction can be predicted using one-class classifier with comparable accuracy to the binary classifiers that use artificially constructed negative examples. Finally, a Bayesian Kernel for SVM was implemented to incorporate the probabilistic information about protein-protein interactions that were compiled from different sources. The probabilistic output from the Bayesian Kernel can assist the biologist to conduct more research on the highly predicted interactions.

ABSTRAK

Matlamat utama pada akhir era genom ialah memahami fungsi protein. Kebiasaannya protein jarang berfungsi sendirian sebaliknya bekerja bersama protein yang lain. Justeru, adalah sangat relevan mengkaji interaksi pasangan protein untuk memahami fungsi protein tersebut. Maka, objektif utama tesis ini adalah untuk meramal interaksi protein-protein berasaskan struktur pertama protein. Dengan menggunakan Mesin Sokongan Vektor (SVM), ciri-ciri protein berlainan dapat dikaji dan diuji. Ciri-ciri ini termasuklah struktur domain protein, hidrophobisiti dan komposisi asid amino. Hasil kajian menunjukan bahawa struktur domain protein mengandungi ciri maklumat yang paling berguna untuk meramal interaksi protein-protein. Tambahan pula, ia memerlukan masa larian yang singkat berbanding ciri-ciri yang lain. Namun demikian, penggunaan SVM binari normal memerlukan sampel data positif dan negatif. Walaupun set data interaksi protein sebagai sampel positif mudah diperolehi, namun tiada pengesahan melalui eksperimen bahawa protein yang tidak-berinteraksi dianggap sebagai sampel negatif. Penyelidik terdahulu mengatasi masalah ini dengan menjana set data pasangan protein yang tidak terkandung dalam Pengkalan Data Interaksi Protein (DIP) secara rawak sebagai sampel negatif. Pendekatan ini boleh digunakan untuk membandingkan ciri-ciri interaksi protein disebabkan ralat yang seragam. Penyelidikan ini menganggap masalah tersebut sebagai masalah pembahagian satu-kelas dan mengatasinya menggunakan SVM Satu-Kelas. SVM Satu-Kelas mencapai ketepatan 80% jika hanya menggunakan sampel positif (pasangan interaksi protein) dalam fasa latihan. Hasil kajian merumuskan bahawa interaksi protein-protein boleh diramal menggunakan pembahagian Satu-Kelas dengan lebih tepat berbanding pengelas binari yang menggunakan binaan buatan sampel negatif. Seterusnya, Bayesian Kernel untuk SVM diimplemetasi bagi menggabungkan kebarangkalian informasi tentang interaksi protein-protein yang telah dikumpul dari pelbagai sumber. Kebarangkalian output dari Bayesian Kernel dapat membantu ahli biologi untuk mengendalikan lebih banyak penyelidikan tentang peramalan interaksi protein.

TABLE OF CONTENTS

CHAPTER	TITLE		PAGE
	ABSTRACT		ii
	ABSTRAK		iii
	TABLE OF CONTENTS		iv
	LIST OF TABLES		vii
	LIST OF FIGURES		ix
	LIST OF ABBREVIATIONS		xii
CHAPTER 1	**INTRODUCTION**		**1**
	1.1	Background of the Problem	1
	1.2	Problem Statement	3
	1.3	The Research Question	3
	1.4	The Goal and Objectives	4
	1.5	Importance of the Study	4
	1.6	Scope of the study	6
	1.7	Thesis Outline	7
	1.8	Summary	9
CHAPTER 2	**BASIC CONCEPTS IN MOLECULAR BIOLOGY**		**10**
	2.1	The Central Dogma of Molecular Biology	10
	2.2	The DNA	12
	2.3	The Proteins	13
	2.4	The Genetic Code	16
	2.5	Proteins Functions	17
	2.6	Protein-Protein Interactions	20
	2.7	Summary	24

CHAPTER 3 LITERATURE REVIEW 25

3.1 Protein Function Prediction 25

3.2 Methods to study protein-protein interactions 30

3.3 Predicting Protein-Protein Interactions 32

3.4 Support Vector Machines 41

3.5 Bayesian Networks 43

3.6 Summary 46

CHAPTER 4 RESEARCH METHODOLGY 47

4.1 Research Design 47

4.2 General Research Framework 48

4.3 Protein Data Sets 51

4.4 Evaluation Measures of the System Performance 54

4.5 Summary 56

CHAPTER 5 COMPARISON OF PROTEIN SEQUENC FEATURES FOR THE PREDICTION OF PROTEIN-PROTEIN INTERACTIONS USING SUPPORT VECTOR MACHINES 57

5.1 Related Work 57

5.2 Comparison Experiment Framework 60

5.3 The Support Vector Mahines 62

5.4 Features Representation 70

5.5 Materials and Implementation 72

5.5.1 Data Sets 72

5.5.2 Data Preprocessing 75

5.6 Results and Discussion 78

5.7 Summary 84

CHAPTER 6 ONE-CLASS SUPPORT VECTOR MACHINES FOR PROTEIN-PROTEIN INTERACTIONS PREDICTION 85

6.1 Related Work 85

6.2 One-Class Classification Problem 88

	6.3	One-Class Support Vector Machines	90
	6.4	Datasets and Implementation	95
	6.5	Results using Domain Feature	97
	6.6	Results using Hydrophobicity Feature	101
	6.7	Discussion	105
	6.8	Summary	106
CHAPTER 7	**REACTIVE CONSTRAINTS PROCESSING ALGORITHMS**		**107**
	7.1	Related Work	107
	7.2	Bayesian Approach	109
		7.2.1 Bayesian Probability	109
		7.2.2 Bayesian Networks	110
	7.3	Kernel Methods	111
	7.4	Bayesian Kernels	117
	7.5	Bayesian Kernel for Protein-Protein Interactions Prediction	119
	7.6	Results and Discussion	121
	7.7	Summary	127
CHAPTER 8	**CONCLUSION AND FUTURE WORK**		**128**
	8.1	Conclusion	128
	8.2	Research Contributions	131
	8.3	Future Work	132
	8.4	Closing	133
	RELATED PUBLICATIONS		134
	REFERENCES		136

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	The amino acids.	14
2.2	The standard genetic code	17
2.3	Proteins functions	18
3.1	Computational methods for protein function prediction	30
3.2	High-throughput experimental approaches to the determination of protein-protein interactions.	33
3.3	Computational methods for protein-protein interactions prediction	36
4.1	The protein interactions of yeast Saccharomyces cerevisiae identified by wet lab experiments.	51
4.2	The contingency table.	54
5.1	The classifier performance on domain structure feature using 10-fold cross validation with variant threshold.	80
5.2	The classifier performance on domain structure with scores feature using 10-fold cross validation with variant threshold.	80
5.3	The classifier performance on hydrophobicity feature using 10-fold cross validation with variant threshold.	81
5.4	The classifier performance on hydrophobicity with scale feature using 10-fold cross validation with variant threshold.	81

5.5	The overall performance of SVM for predicting PPI using domain and hydrophobicity features.	82
6.1	One-Class performance using different kernel with the domain feature	101
6.2	One-Class performance using different kernel with the domain feature.	104
7.1	Bayesian Kernel performance with varied threshold using domain feature.	123
7.2	Bayesian Kernel performance compared to the standard kernels using domain feature.	124
7.3	Performance comparison with the cited literature.	104

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	The central dogma of molecular biology (Korf et al., 2003).	11
2.2	Schematic drawing of protein secondary structure (Punta and Rost, 2005).	15
2.3	The protein-protein interaction network in yeast (Uetz, 2000).	21
2.4	(a) Large protein complex and its protein-protein interactions. (b) The schematic interaction (Cramer et al., 2001).	22
3.1	Different methods for inferring protein function.	26
3.2	The overlap between different high-throughput experiments.	34
4.1	The general research operational framework.	50
4.2	A simplified entity-relationship diagram of the DIP database (Xenarios et al., 2002).	53
5.1	The framework of comparing protein sequence features. threshold.	61
5.2	(a) A separating hyperplane with small margin. (b)A separating hyperplane with larger margin.	62

5.3	(a) Hard margin solution when data contain noise. (b) Soft margin solution when data contain noise.	68
5.4	Part of the protein-protein interactions list from DIP.	73
5.5	Part of the protein-protein interactions list with sequences name only.	74
5.6	Part of the protein sequence file.	75
5.7	Part of the protein domains file.	76
5.8	Part of the protein domains structure for the yeast genome.	77
5.9	Part of the training data file.	77
5.10	The ROC curves and scores for predicting protein-protein interactions.	83
6.1	Target and outlier classes in the one-class classification problem.	89
6.2	Classification in the one-class SVM.	93
6.3	The implementation framework for the one-class SVM.	96
6.4	The one-class SVM performance using domain feature with the linear kernel.	99
6.5	The one-class SVM performance using domain feature with the polynomial kernel.	99
6.6	The one-class SVM performance using domain feature with the RBF kernel.	100
6.7	The one-class SVM performance using domain feature with the sigmoid kernel.	100

6.8	The one-class SVM performance using hydrophobicity feature with the linear kernel.	102
6.9	The one-class SVM performance using hydrophobicity feature with the polynomial kernel.	103
6.10	The one-class SVM performance using hydrophobicity feature with the RBF kernel.	103
6.11	The one-class SVM performance using hydrophobicity feature with the sigmoid kernel.	104
7.1	Illustration of mapping input data to a feature space.	113
7.2	The ROC curve for the Bayesian kernel and the standards kernel.	124
7.3	The distribution of the probabilistic output for the Bayesian kernel	104

LIST OF ABBREVIATIONS

BN	–	Bayesian Networks
DIP	–	The Database of Interacting Proteins
DNA	–	Deoxyribonucleic Acid
DPEA	–	Domain Pair Exclusion Analysis
DPI	–	Dual Polarisation Interferometry
FNR	–	False Negative Rate
FPR	–	False Positive Rate
GO	–	Gene Ontology
HGP	–	Human Genome Project
InterDom	–	The Database of Interacting Domains
KBM	–	Kernel Based Methods
KM	–	Kernel Matrix
LIBSVM	–	Library for Support Vector Machine
MIPS	–	Munich Information Center for Protein Sequences
mRNA	–	Messenger Ribonucleic Acid
MSSC	–	Maximum Specificity Set Cover
ncRNA	–	Non-coding Ribonucleic Acid
PDB	–	Protein Data Bank
PFAM	–	Protein Families Database
PID	–	Potentially Interacting Domain
PIE	–	Probabilistic Interactome Experimental
PPI	–	Protein-Protein Interactions
QP	–	Quadratic Programming
RBF	–	Radial Basis Function
RNA	–	Ribonucleic Acid
RNAi	–	Ribonucleic Acid inference mechanism

ROC	–	Receiver Operating Characteristic
RVM	–	Relevance Vector Machine
SGD	–	Saccharomyces Genome Database
SVM	–	Support Vector Machines
TAP	–	Tandem Affinity Purification
TPR	–	True Positive Rate
YPD	–	The Yeast Proteome Database

CHAPTER 1

INTRODUCTION

Bioinformatics or computational biology is broadly defined as the application of computational techniques to solve biological problems. This field has arisen in parallel with the development of automated high throughput methods of biological and biochemical discovery that yield a huge and variety forms of experimental data, such as DNA and protein sequences, gene expression patterns, and chemical structures. Major research efforts in bioinformatics include sequence alignment, gene finding, genome assembly, protein structure alignment, protein structure prediction and prediction of protein-protein interactions. In this thesis, the prediction of protein-protein interactions from sequences data using machine learning techniques is presented. The background of the problem, objectives, importance of the study, and the scope of this research is presented and discussed in this chapter.

1.1 Background of the Problem

The majority of functions in cells are accomplished by proteins. Therefore, assigning functions to the proteins encoded by a genome is one of the crucial steps in gaining understanding of the organism. Because the function of half of all proteins in newly sequenced genomes often is completely unknown, complete genome sequencing gives much less insight into the organism than initially hoped for (Walhout and Vidal, 2001). Although, most methods annotating protein function

utilise sequence homology to proteins of experimentally known function, such a homology-based annotation transfer is problematic and limited in scope. Therefore, alternative approaches have begun to develop. These approaches include methods based on phylogenetic patterns, gene expression, and protein-protein interactions data.

The sequencing of entire genomes has moved the attention from the study of single proteins or small complexes to that of the entire proteome. Most proteins do not function in isolation, but collaborate with other proteins. In this context, identifying protein-protein interactions (PPI) is an important goal of proteomics. Protein-protein interactions data can help researchers to infer protein's functions based on the information available about its partner. Usually, laboratory experiments are used such as yeast two-hybrid analysis, protein microarrays and immunoaffinity chromatography followed by mass spectrometry. Recently, computational methods have been introduced because laboratory experiments are costly, time-consuming and suffer from high false positive rates.

Part of the reason why it is difficult to relate the chemical function of a protein to its biological purpose using homology-based annotation is that proteins do not function alone. To understand the function of a protein, it must be considered in its proper cellular context, for example by appreciating how the cell would behave without it (Attwood and Miller, 2001). Many proteins are parts of larger complexes, which are the functional units that fulfill a role in the cell (Gavin *et al*., 2002). In this regard it can be argued that knowing proteins partners can give important clue about its function. Therefore it is highly relevant to study the interaction partners of a protein in order to understand its function (Ho *et al*., 2002) (Deng *et al*., 2002).

Most protein-protein interactions have been discovered by laboratory techniques such as yeast two-hybrid system that can detect all possible combinations of interactions. However, these findings can be superfluous and the number of experimentally determined structures for protein-protein interactions is still quite small. As a result, methods for computational prediction of protein-protein interactions are becoming increasingly important.

Therefore the aim of this research is to predict protein-protein interactions from protein primary structure data using machine learning techniques. Then the availability of both the experimental and the predicted protein-protein interactions data can be used to construct more reliable dataset for the prediction of proteins functions.

1.2 Problem Statement

The research problem that we are trying to solve in this research can be described as following. Given the protein-protein interactions data for the budding yeast, *Saccharomyces cerevisiae* that are listed in the Database of Interacting Proteins (DIP) and its protein sequences data, it is a challenging task to accurately predict new protein-protein interactions based on that data using machine learning techniques.

1.3 The Research Question

The main research question is:

How can the protein-protein interactions be predicted from protein sequences data using machine learning techniques?

Thus, the following issues will arise to answer the main research question stated above:

- How to identify the best protein sequence features that can be used to train the learning algorithm?
- How to overcome the unavailability of confirmed non-interacting proteins which is important as negative examples for the training of the learning algorithm?

- How to incorporate the probabilistic protein-protein interactions information to improve the prediction accuracy?

1.4 The Goal and Objectives

The main goal of this research is to develop a computational technique using the Support Vector Machines (SVM) and Bayesian approach to predict protein-protein interactions form protein sequences data of the budding yeast, *Saccharomyces cerevisiae*.

To achieve this goal the following objectives have been set:

- To investigate different protein sequence features for the prediction of protein-protein interactions using the support vector machines.
- To formulate the problem of predicting protein-protein interactions as a one-class classification problem then solve it using the One-Class SVM
- To incorporate the probabilistic protein-protein interactions information using Bayesian kernel.
- To test, evaluate, and enhance the prediction system.

1.5 Importance of the Study

Assigning functions to the proteins encoded by a genome is one of the crucial steps in gaining understanding of the organism. Besides, the study of protein function is fundamental to the drug discovery process. However, the function of half of all proteins in newly sequenced genomes often is completely unknown (Walhout and Vidal, 2001). Therefore, assigning function to the newly discovered proteins represents a major challenge in the post-genomic era, and could help biologists to better understand the molecular mechanisms of biological events.

The most common approach to identify protein function is based on sequence similarity. However, about 30% to 40% of the newly discovered proteins can not be assigned function based on sequence homology or similarity because they do not have statistically significant similarity with known protein (Letovsky and Kasif, 2003).

Inferring protein function can be made via protein-protein interaction studies. This is due to the fact that proteins work in a context of other proteins and rarely work alone. Hence, the function of unknown protein may be discovered if information about its interaction partners of known function is available. For that reason, the study of protein interactions has been fundamental to the understanding of how proteins function within the cell. Characterizing the interactions of proteins in a given cellular proteome will be the next milestone along the road to understanding the biochemistry of the cell. As a result, studying protein-protein interactions to gain insight on protein functions has become a topic of enormous interest in recent years, resulting many efforts devoted to its research.

The interactions between proteins are important for many biological functions. Almost all processes in of molecular biology are affected by protein-protein interactions (Alberts *et al*. 2002, Lodish *et al*. 2004). Replication, transcription, translation, signal transduction, protein trafficking, and protein degradation are all accomplished by protein complexes, often temporally assembled and disassembled to accomplish vital processes. In fact, the importance of protein–protein interactions in the post-genomic era is becoming more noticeable due to the huge volume of data that became available. Hence, studying protein-protein interactions is crucial to gain insight on protein functions of the newly sequenced genomes.

Until recently, information about protein–protein interactions was gathered via experiments that were individually designed to identify and validate a small number of specifically targeted interactions (Legrain *et al*., 2001). This type of experiments is called small-scale experiments. This traditional source of information has been increased recently by the results of high-throughput experiments designed to exhaustively explore all the potential interactions within entire genomes.

However, the many discrepancies between the interacting partners identified in high-throughput studies and those identified in small-scale experiments highlight the need for caution when interpreting results from high-throughput studies (Salwinski and Eisenberg 2003).

These discrepancies represent the need for the development of computational methods for data validation. Indeed, the interaction data that have been provided by high throughput technologies like the yeast two-hybrid system are known to suffer from many false positives. In addition, *in vivo* experiments elucidating protein-protein interactions are still time-consuming and labor-intensive methods. As a result, complementary computationally methods capable of accurately predicting interactions would be of considerable value. Furthermore, computational methods for the prediction of protein interactions will provide more data which will enable predicting protein function more precisely since the function of proteins with three or more partners can be more accurately predicted.

1.6 Scope of the Study

This study will focus on predicting protein-protein interactions from protein sequence information of the Yeast, *Saccharomyces cerevisiae* genome. The protein interactions dataset was obtained from the Database of Interacting Proteins (DIP) and the protein sequences data was obtained from Munich Information Center for Protein Sequences (MIPS). The DIP database was developed to store and organize information on binary protein–protein interactions that was retrieved from individual research articles (Xenarios *et al.*, 2002). The DIP database provides sets of manually curated protein-protein interactions in Saccharomyces cerevisiae. The current version contains 4749 proteins involved in 15675 interactions for which there is domain information. DIP also provides a high quality core set of 2609 yeast proteins that are involved in 6355 interactions which have been determined by at least one small-scale experiment or at least two independent experiments and predicted as positive by a scoring system (Deane *et al.*, 2001).

However, it should be noted that protein-protein interactions are sometimes confused with metabolic pathways. Metabolic Pathway is a series of enzyme-catalyzed reactions. Each reaction produces a product which becomes the substrate for the next reaction. Although the structures of metabolic pathways and protein interaction maps are similar, there are a number of significant differences: While metabolic pathways focus on the conversion of small molecules and the enzymes responsible for these conversions, protein interaction maps concentrate mainly on physical contacts without obvious chemical conversions. Physical interactions are certainly of great utility when one studies single proteins or defined biological processes, but themselves do not reflect the huge amount of knowledge that has been accumulated in the biological literature. In this research we only attempt to predict physical protein-protein interactions.

1.7 Thesis Outline

The outline and the flow of the contents of this thesis can be described as follows:

- The thesis begins with Chapter 1 in which this section is part of it. The chapter explains the key concepts, introducing the problem of this research, list the objectives, and determine the scope of this work.

- Chapter 2 reviews and explains the basic terms and concepts in the molecular biology such as the central dogma of molecular biology, DNA, and proteins. It also examines amino acids and proteins in terms of their nature, formation, structure and their importance.

- The following is Chapter 3 which discuss and overview protein function prediction and protein-protein interactions prediction methods. This chapter begins by reviewing several approaches to protein function prediction and its relation to protein-protein interactions. Then it describes the experimental techniques that are being used to discover and identify protein-protein interactions and

highlights the need for computational approaches. It also reviews the computational methods that have been developed to predict protein-protein interactions..

- Chapter 4 describes the overall methodology adopted in this research to achieve the objectives of this thesis.

- Chapter 5 presents and discusses the process of using support vector machines (SVM) to predict protein-protein interactions for protein sequence information. Using SVM, different protein sequence features have been studied and examined. These features include protein domain structures, hydrophobicity and amino acid compositions. At the end of this chapter, the results of studying and comparing these features are presented.

- Chapter 6 shows how the problem of predicting protein-protein interactions can be modeled as a one-class classification problem. It also present the One-Class SVM classifier and its implementation to prediction protein-protein interactions as a one-class classification problem using only positive examples (interacting protein pairs) in training phase. At the end of this chapter, the results of using the One-Class SVM are presented.

- Chapter 7 describes the implementation of Bayesian Kernel for SVM to predict protein-protein interactions. Bayesian Kernel for SVM was implemented to incorporate the probabilistic information about protein-protein interactions that were compiled from different sources. This chapter also shows that the probabilistic output from the Bayesian Kernel can assist the biologist to conduct more research on the highly predicted interactions

- Chapter 9 concludes and summarizes this thesis, highlights the contributions and findings of this work, and provides suggestions and recommendations for future research.

1.8 Summary

The aim of this chapter is to give a broad overview of the problem of protein functions predictions and protein-protein interactions prediction and the general methods to solve it. This chapter serves as an introductory text to the research problem addressed in this thesis. The goal, objectives, the scope, and the organization of the thesis were presented. However, we have not presented a comprehensive review of the methods that have been employed to predict protein-protein interactions. The next chapter (Chapter 2) describes the basic concepts of molecular biology then the following chapter (Chapter 3) surveys the previous research that relates most closely to this work in.

CHAPTER 2

BASIC CONCEPTS IN MOLECULAR BIOLOGY

For a better understanding of this research, an introductory chapter to the basic concepts and terminology of molecular biology and biological sequence analysis is inevitable. This chapter begins with a brief description of the central dogma of molecular biology which involves the production of proteins from DNA. Then an overview of protein's definition, nature, structure and its importance is presented. The chapter also explains the composition of proteins and its building blocks, the amino acids. In addition to this chapter, a glossary of biological terms is offered in Appendix A.

2.1 The Central Dogma of Molecular Biology

The central dogma of molecular biology is based on the assumption that each gene in the deoxyribonucleic acid (DNA) molecule carries the information needed to construct one protein. DNA is a nucleic acid that contains the genetic instructions for the development and function of living organisms (Alberts *et al*., 2002). All known cellular life and some viruses contain DNA. The main role of DNA in the cell is the long term storage of information. It is often compared to a blueprint, since it contains the instructions to construct other components of the cell, such as proteins and ribonucleic acid (RNA) molecules. The DNA segments that carry genetic information are called genes, but other DNA sequences have structural purposes, or are involved in regulating the expression of genetic information.

The central dogma involves two steps: *transcription* and *translation*. Transcription produces an mRNA (messenger RNA) sequence using the DNA sequence as a template. The subsequent process, called *translation*, synthesizes the protein according to information coded in the mRNA (Korf *et al*., 2003). This process is performed by sub cellular elements called *ribosomes*. Proteins are created in the nucleus of all cells in a living organism. The DNA in each cell provides a recipe of how and when proteins should be created. The process in which proteins are created is called *protein synthesis*. This process is illustrated in Figure 2.1.

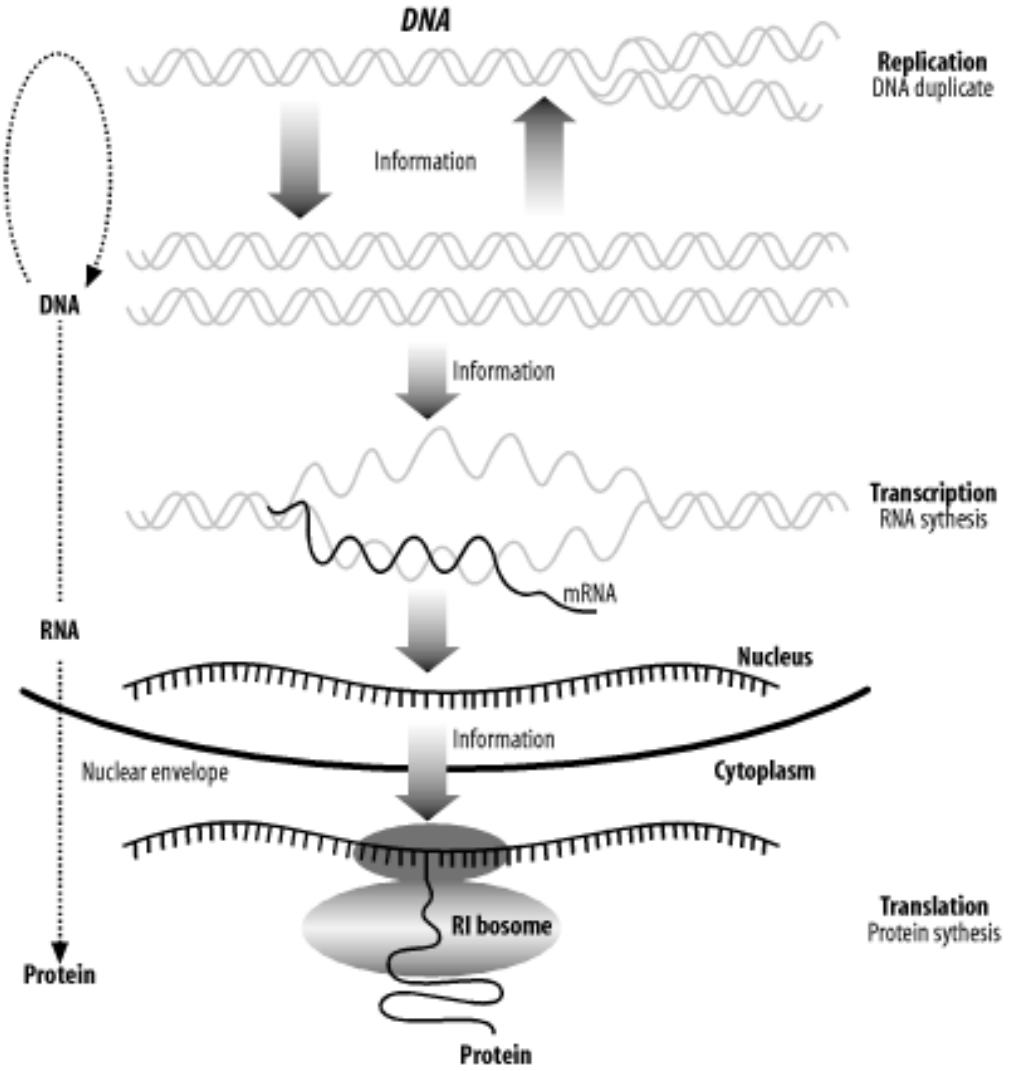


Figure 2.1: The central dogma of molecular biology (Korf *et al*., 2003).

2.2 The DNA

As mentioned earlier, the hereditary material that carries the blueprint for an organism from one generation to the next is called deoxyribonucleic acid (DNA). Every time cells divide, the DNA is duplicated in a process called DNA replication. The entire DNA of an organism is called its genome. Understanding the various organisms' genomes is one of the most important challenges in the post-genomic era (Palsson, 2000). Modern medicine, agriculture, and industry will increasingly depend on the knowledge of genomes to develop individualized medicines that select and modify the most desirable traits in plants and animals, and understand the relationships among species.

The alphabet of the DNA language is simple, consisting of just four nucleotides: adenine, cytosine, guanine, and thymine. For simplicity, they are abbreviated as A, C, G, and T. DNA usually exists as a double-stranded molecule, but generally just one strand at a time is referred. The pairing rule of DNA is that A pairs with T, and C pairs with G. Hence, it is very easy to determine the sequence of the complementary strand of any DNA sequence. Here's an example of a DNA sequence with its complementary sequence:

G A T T A G C T C C A G G A A T
C T A A T C G A G G T C C T T A

DNA has polarity with its ends are referred to as 5-prime (5´) and 3-prime (3´). This nomenclature comes from the chemical structure of DNA. While it isn't necessary to understand the chemical structure, the terminology is important. For example, "the 5´ end of the gene," means the beginning of the gene. Usually DNA sequence is displayed left to right, and the convention is that the left side is the 5´ end and the right side is the 3´ end.

A gene is a functional unit of the genome (the full DNA sequence of an organism). Most genes contain instructions for producing proteins at a certain time and in a certain space. Some genes have very narrow windows of activity, while others are everywhere. However, not all genes code for proteins. Some genes

produce RNAs that aren't translated into proteins and are therefore called noncoding RNAs (ncRNA) (Korf *et al*., 2003).

DNA doesn't encode proteins on its own. DNA is copied into RNA by a protein called RNA polymerase in a process called transcription. Chemically, RNA is a lot like DNA except that it uses uracil instead of thymine and it is single stranded instead of double stranded. The RNA alphabet is A, C, G, and U, and an RNA molecule might look like this:

G A A U U G C U C C A G G A A U

If the RNA transcript from a gene is a transfer RNA (tRNA), ribosomal RNA (rRNA), or other ncRNA, it may undergo some chemical modifications, but the gene product remains as an RNA molecule. RNAs corresponding to protein coding genes are called messenger RNAs (mRNA).

2.3 The Proteins

A protein is linear polymer of amino acids linked together by peptide bonds. There are twenty amino acids that compose the standard chemical alphabet used to build proteins. The amino acids are small molecules that share a common motif, of three substitute chemical groups arranged around a central carbon atom. One of the substitute groups is always an amino group; another is always carboxylic acid group. The average protein size is around 200 amino acids long, while large proteins can reach over a thousand amino acids.

The protein alphabet contains 20 symbols, A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y. The names, abbreviations, and structures of the amino acids are shown in Table 2.1.

Table 2.1: The amino acids.

Amino acid	Abbreviation	Symbol	Properties
Alanine	Ala	A	Hydrophobic
Cysteine	Cys	C	Neutral; forms disulfide bridges
Aspartate	Asp	D	Negatively charged
Glutamate	Glu	E	Negatively charged
Phenylalanine	Phe	F	Hydrophobic; aromatic
Glycine	Gly	G	Neutral; smallest amino acid
Histidine	His	H	Positively charged; aromatic
Isoleucine	Ile	I	Hydrophobic
Lysine	Lys	K	Positively charged
Leucine	Leu	L	Hydrophobic
Methionine	Met	M	Hydrophobic; start amino acid
Asparagine	Asn	N	Neutral ; hydrophilic
Proline	Pro	P	Hydrophobic
Glutamine	Gln	Q	Neutral ; hydrophilic
Arginine	Arg	R	Positively charged
Serine	Ser	S	Neutral; hydrophilic
Threonine	Thr	T	Neutral ; hydrophilic
Valine	Val	V	Hydrophobic
Tryptophan	Trp	W	Hydrophobic; aromatic
Tyrosine	Tyr	Y	Hydrophobic; aromatic

Using one-letter symbols, a protein sequence might be written like this:

M L V G S R A

The sequences of proteins are one-dimensional, but their shapes are three-dimensional. Proteins can fold into very specific three-dimensional shapes that are dependent on their amino acid sequences. Thus, the amino acid sequence determines the shape of the protein and the shape determines the function. Therefore, while

DNA and RNA are largely used to store and send information, proteins carry out almost all processes in the cell. Also, proteins determine the shape and structure of the cell, and also serve as the main instruments of molecular recognition and catalysis.

Although proteins have many different shapes and sizes, if we look closely at the structure, we can find recurring structural themes that biologists call secondary structure. The most common themes are the α-helix, β-sheet, and random coil. In Figure 2.2, these themes are represented as cylinders, arrows, and squiggly lines.

Figure 2.2: Schematic drawing of protein secondary structure (Punta *et al*., 2005).

When the sequences of primary structures tend to arrange themselves into regular formations, these units are referred to as secondary structure. The angles and hydrogen bond patterns between backbone atoms are determinant factors in protein

secondary structure. Secondary structure is subdivided into three parts: alpha-helix, beta-sheet and loop.

Alpha-helix is spiral turns of amino acids while a beta-sheet is flat segments or strands of amino acids formed usually by a series of hydrogen bonds. Beta-strands are the most regular form of extended polypeptide chain in protein structures. Loops usually serve as connection points between alpha-helices and beta-sheets. The do not have patterns like alpha-helices and beta-sheets and they could be any other part of the protein structure. They are sometimes known as random coil.

2.4 The Genetic Code

The information in DNA and RNA is translated to protein sequence using a complex machine composed of proteins and ncRNAs called the ribosome reads an mRNA sequence and writes a protein sequence. The mRNA is read three nucleotides at a time. The nucleotide triplets are called codons. Each codon corresponds to a single amino acid. The mapping from codons to amino acids is called the genetic code. The genetic code is one of the universal laws of molecular biology.

Because codons are three nucleotides long and there are four possible nucleotides at each position, it follows that there are 64 (4^3) possible codons. However, there are only 20 amino acids. Therefore there is a redundancy in the genetic code. Table 2.2 shows the standard nuclear genetic code. It can be observed from Table 2.2 that there is a pattern in the genetic code redundancies. For example, the third position of a codon is often insignificant; A, C, G, or T all lead to the same translation. When this isn't the case, A and G are usually synonymous, as are C and T. A and G belong to the same chemical class, called purines, and C and T belong to another class, called pyrimidines. In addition to the amino acids, there are three stop codons. When a ribosome catches a stop codon, translation terminates, and the protein is released. All proteins start with the amino acid methionine. This has only one codon, ATG, and so ATG is often called the start codon.

Table2.2: The standard genetic code.

		Second Position					
		T	**C**	**A**	**G**		
First Position	**T**	TTT Phe (F) TTC Phe (F) TTA Leu (L) TTG Leu (L)	TCT Ser (S) TCC Ser (S) TCA Ser (S) TCG Ser (S)	TAT Tyr (Y) TAC TAA STOP TAG STOP	TGT Cys (C) TGC TGA STOP TGG Trp (W)	T C A G	**Third Position**
	C	CTT Leu (L) CTC Leu (L) CTA Leu (L) CTG Leu (L)	CCT Pro (P) CCC Pro (P) CCA Pro (P) CCG Pro (P)	CAT His (H) CAC His (H) CAA Gln (Q) CAG Gln (Q)	CGT Arg (R) CGC Arg (R) CGA Arg (R) CGG Arg (R)	T C A G	
	A	ATT Ile (I) ATC Ile (I) ATA Ile (I) ATG Met (M)	ACT Thr (T) ACC Thr (T) ACA Thr (T) ACG Thr (T)	AAT Asn (N) AAC Asn (N) AAA Lys (K) AAG Lys (K)	AGT Ser (S) AGC Ser (S) AGA Arg (R) AGG Arg (R)	T C A G	
	G	GTT Val (V) GTC Val (V) GTA Val (V) GTG Val (V)	GCT Ala (A) GCC Ala (A) GCA Ala (A) GCG Ala (A)	GAT Asp (D) GAC Asp (D) GAA Glu (E) GAG Glu (E)	GGT Gly (G) GGC Gly (G) GGA Gly (G) GGG Gly (G)	T C A G	

2.5 Proteins Functions

Proteins are the main players within the cell, known to be carrying out the duties specified by the information encoded in genes (Lodish *et al.*, 2004). Proteins compose half the dry weight of a cell, while other macromolecules such as DNA and RNA compose only 3% and 20% respectively (Voet and Voet, 2004). The total

complement of proteins expressed in a particular cell or cell type at a given time point or experimental condition is known as its proteome.

The main characteristic of proteins that enables them to carry out their diverse cellular functions is their ability to bind other molecules specifically and tightly. The region of the protein responsible for binding another molecule is known as the binding site and is often a depression or "pocket" on the molecular surface (Lodish *et al.*, 2004). This binding ability is mediated by the tertiary structure of the protein, which defines the binding site pocket, and by the chemical properties of the surrounding amino acids' side chains.

Proteins can bind to other proteins as well as to small-molecule substrates. When proteins bind specifically to other copies of the same molecule, they can oligomerize to form fibrils. Protein-protein interactions also regulate enzymatic activity, control progression through the cell cycle, and allow the assembly of large protein complexes that carry out many closely related reactions with a common biological function. Proteins can also bind to, or even be integrated into, cell membranes. The ability of binding partners to induce conformational changes in proteins allows the construction of enormously complex signaling networks. Table 2.3 summarizes the different proteomic functions. The following paragraphs describe some of these functions briefly.

Table 2.3: Proteins functions.

Protein Function	**Description**	**Examples**
Catalytic proteins (Enzymes)	Catalyze reactions in cell	Lactase Protein kinase RNase Chymotrypsin
Regulatory proteins	Modulate biological activity	Insulin DNA-binding proteins
Defense proteins	Protect organism	Immunoglobulins Antibiotics

Transport proteins	Bind and carry specific molecules or ions	Hemoglobin
Structural Proteins	Support or strengthen biological structures	Collagen–tendon Cartilage Leather
Nutrient/Storage proteins	Source of amino acids	Ovalbumin–egg white Casein–milk

The best-known function or role of proteins in the cell is their duty as enzymes, which catalyze chemical reactions. Enzymes are usually highly specific catalysts that accelerate only one or a few chemical reactions. Enzymes affect most of the reactions involved in metabolism and catabolism as well as DNA replication, DNA repair, and RNA synthesis. Some enzymes act on other proteins to add or remove chemical groups in a process known as post-translational modification. About 4,000 reactions are known to be catalyzed by enzymes (Bairoch, 2000).

Many proteins are involved in the process of cell signaling and signal transduction. Some proteins, such as insulin, are extra-cellular proteins that transmit a signal from the cell in which they were synthesized to other cells in distant tissues. Others are membrane proteins that act as receptors whose main function is to bind a signaling molecule and induce a biochemical response in the cell.

Antibodies are protein components of adaptive immune system whose main function is to bind antigens, or foreign substances in the body, and target them for destruction. Antibodies can be secreted into the extra-cellular environment or anchored in the membranes of specialized B cells known as plasma cells. While enzymes are limited in their binding affinity for their substrates by the necessity of conducting their reaction, antibodies have no such constraints. An antibody's binding affinity to its target is extraordinarily high.

Many ligand transport proteins bind particular small biomolecules and transport them to other locations in the body of a multicellular organism. These proteins must have a high binding affinity when their ligand is present in high concentrations but must also release the ligand when it is present at low concentrations in the target tissues. The canonical example of a ligand-binding protein is haemoglobin, which transports oxygen from the lungs to other organs and tissues in all vertebrates and has close homologs in every biological kingdom.

Structural proteins confer stiffness and rigidity to otherwise fluid biological components. Most structural proteins are fibrous proteins; for example, actin and tubulin are globular and soluble as monomers but polymerize to form long, stiff fibers that comprise the cytoskeleton, which allows the cell to maintain its shape and size. Collagen and elastin are critical components of connective tissue such as cartilage, and keratin is found in hard or filamentous structures such as hair, nails, feathers, hooves, and some animal shells.

2.6 Protein-Protein Interactions

Protein-protein interactions refer to the association of protein molecules and the study of these associations from the perspective of biochemistry, signal transduction and networks. Proteins might interact for a long time to form part of a protein complex or a protein may interact briefly with another protein just to modify it (for example, a protein kinase will add a phosphate to a target protein).

Protein-protein interactions are essential to virtually every cellular process (Phizicky and Fields, 1995). For example, signals from the exterior of a cell are mediated to the inside of that cell by protein-protein interactions of the signaling molecules. This process, called signal transduction, plays a fundamental role in many biological processes and in many diseases (e.g. cancer).

It has been proposed that all proteins in a given cell are connected in a huge network in which certain protein interactions are forming and dissociating constantly

(Bork *et al*., 2004). An interaction map of the yeast proteome assembled from published interactions is shown in Figure 2.3. The map contains 1,548 proteins (boxes) and 2,358 interactions (connecting lines) (Schwikowski *et al.*, 2000).

It is also estimated that even simple single-celled organisms such as yeast have their roughly 6000 proteins interact by at least 3 interactions per protein, i.e. a total of 20,000 interactions or more. By extrapolation, there may be on the order of ~100,000 interactions in the human body.

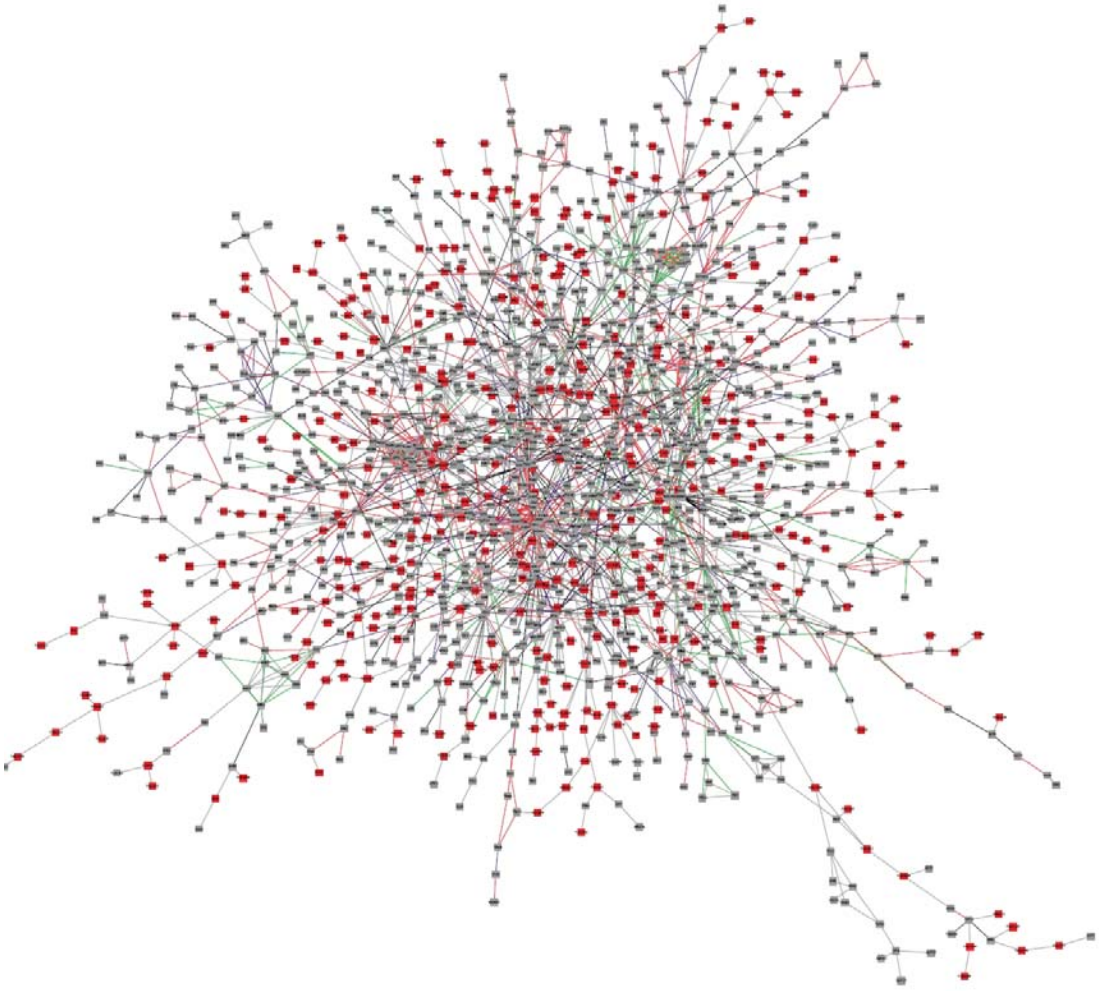
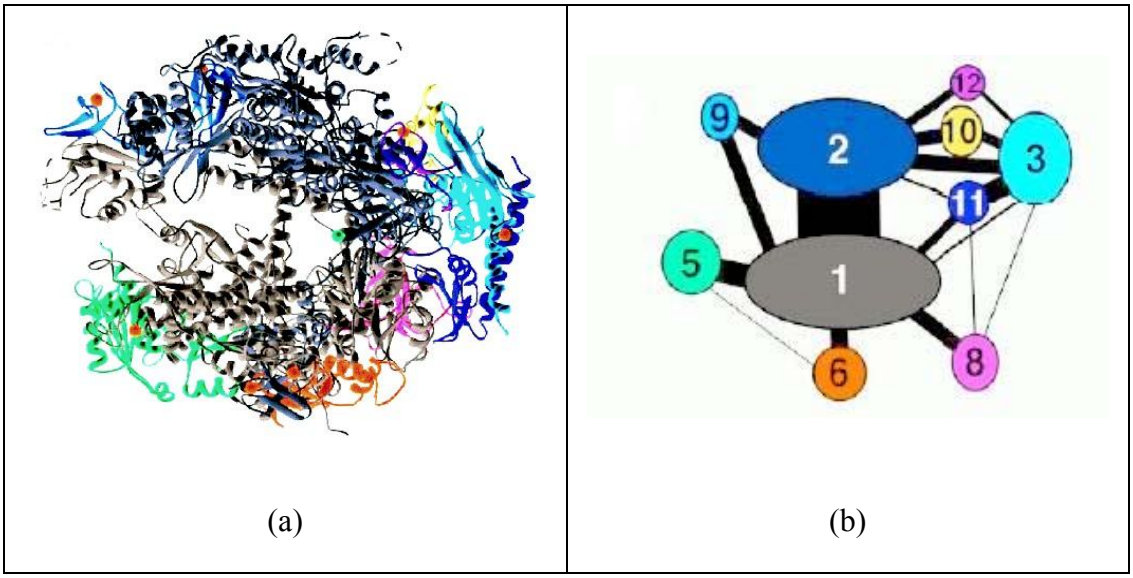


Figure 2.3: The protein-protein interaction network in yeast (Schwikowski *et al*., 2000).

Any listing of major research topics in biology - for example, DNA replication, transcription, translation, splicing, secretion, cell cycle control, signal transduction, and intermediary metabolism - is also a listing of processes in which protein complexes have been implicated as essential components (Phizicky and Fields, 1995). Figure 2.4 shows Ribosomes or RNA polymerases as an example for protein-protein interactions in a multi-protein complex. The schematic interaction diagram for the 10 subunits in RNA polymerases complex is shown in Figure 2.4 (b).



(a) (b)

Figure 2.4: (a) Large protein complex and its protein-protein interactions. (b) The schematic interaction (Cramer *et al*., 2001).

Protein-protein interactions can be classified based on the proteins involved (structural or functional groups) or based on their physical properties (weak and transient vs. strong and permanent). Protein interactions are usually mediated by defined domains. Hence interactions can also be classified based on the underlying domains.

Experimentally, interactions between pairs of proteins can be detected from yeast two-hybrid systems, from affinity purification/mass spectrometry assays, or from protein microarrays. In parallel to the experimental determination of the protein-protein interactions, computational methods are being developed. Protein-

protein interaction prediction is a field combining computational techniques and structural biology in an attempt to identify and catalog interactions between pairs or groups of proteins.

Forces that mediate protein-protein interactions include electrostatic interactions, hydrogen bonds, the van der Waals attraction and hydrophobic effects. The average protein-protein interface is not less polar or more hydrophobic than the surface remaining in contact with the solvent. Water is usually excluded from the contact region. Non-obligate complexes tend to be more hydrophilic in comparison, as each component has to exist independently in the cell.

It has been proposed that hydrophobic forces drive protein-protein interactions and hydrogen bonds and salt bridges confer specificity (Young *et al.*, 1994). Van der Waals interactions occur between all neighbouring atoms, but these interactions at the interface are no more energetically favorable than those made with the solvent. However, they are more numerous, as the tightly packed interfaces are denser than the solvent and hence they contribute to the binding energy of association.

Hydrogen bonds between protein molecules are more favourable than those made with water. Interfaces in permanent associations tend to have fewer hydrogen bonds than interfaces in non-obligate associations. Interfaces have been shown to be more hydrophobic than the exterior but less hydrophobic than the interior of a protein. Permanent complexes have interfaces that contain hydrophobic residues, whilst the interfaces in non-obligate complexes favour the more polar residues (Koike and Takagi, 2003).

Most of the interactions data have been identified by high-throughput technologies like the yeast two-hybrid system, which are known to yield many false positives (Kim *et al*., 2002). In addition, in vivo experiments that identify protein-protein interaction are still time-consuming and labor-intensive; besides, they identify a small number of interactions. As a result, methods for computational prediction of protein-protein interactions based on sequence information are becoming increasingly important.

2.7 Summary

In this chapter, various concepts in molecular biology have been presented. This is essential to facilitate better understanding of the research discussed in this thesis. In conclusion, protein-protein interactions are of central importance for virtually every process in a living cell. Information about these interactions improves our understanding of diseases and can provide the basis for new therapeutic approaches.

CHAPTER 3

LITERATURE REVIEW

Related research in the field of computational prediction of protein-protein interactions is presented in this chapter. This chapter begins by reviewing several approaches to protein function prediction and its relation to protein-protein interactions. After that it describes the experimental techniques that are being used to determine and identify protein-protein interactions and highlights the need for computational approaches. Then it reviews the research that has been done to computationally predict protein-protein interactions. At the end, a summary of the literature review is presented.

3.1 Protein Function Prediction

The field of bioinformatics has arisen in parallel with the development of automated high throughput methods of biological and biochemical discovery that yield a variety of forms of experimental data, such as DNA sequences, gene expression patterns, and chemical structures. One of the major challenging tasks in bioinformatics is to infer and predict the function of the newly discovered proteins.

Proteins carry out the majority of tasks in organisms, such as catalysis of biochemical reactions, transport of nutrients, recognition and transmission of signals. The role of any particular protein is referred to as its function. However, protein function is not a well-defined term; instead function is a complex phenomenon that is

associated with many mutually overlapping levels: biochemical, cellular, organism mediated, developmental, and physiological. Thus, the determination of protein functions is a complex problem in bioinformatics research. The sequencing of entire genomes has moved the attention from the study of single proteins or small complexes to that of the entire proteome (Hodgman, 2000).

One of the most fundamental tools in the field of bioinformatics is sequence alignment. By aligning sequences to one another, it is possible to evaluate how similar the sequences are and identify conserved regions in sets of related sequences. This is used extensively to assign function to genes in newly sequenced genomes. Although, most methods annotating protein function utilise sequence homology to proteins of experimentally known function, such a homology-based annotation transfer is problematic and limited in scope. Therefore, researchers have begun to develop different methods that predict protein function, including phylogenetic patterns, gene expression, and protein-protein interactions. Figure 3.1 shows different approaches to infer and predict protein function.

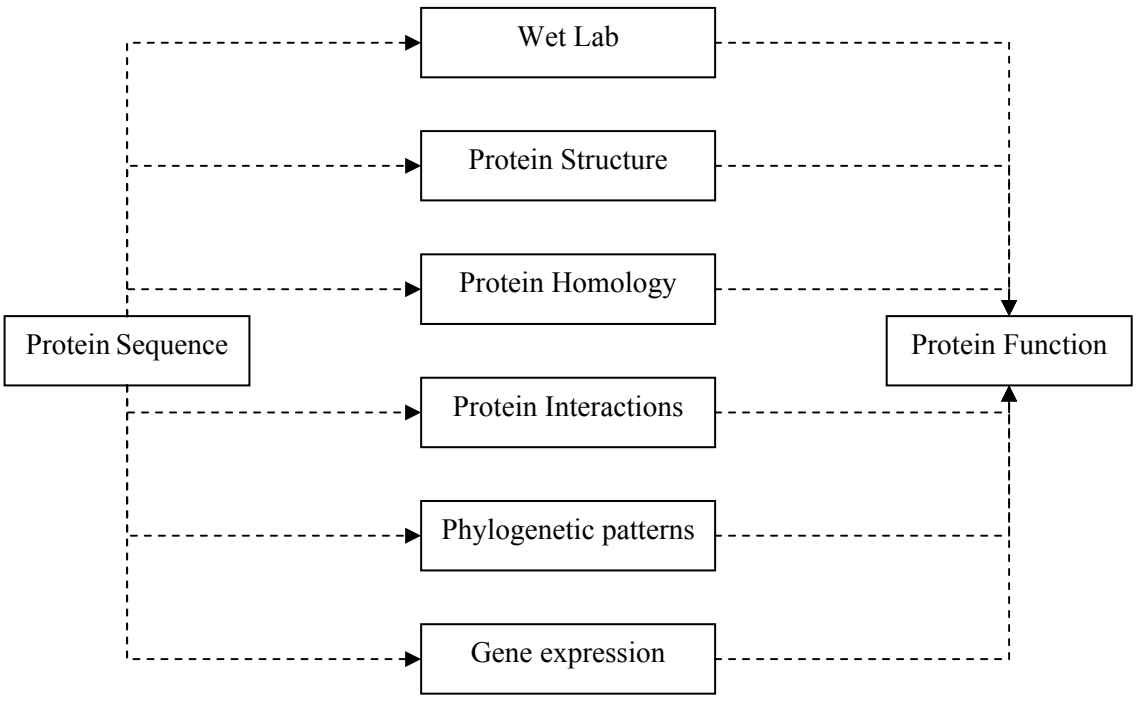


Figure 3.1: Different methods for inferring protein function.

The Yeast Protein Database (YPD) lists 6281 proteins with 3854 being annotated, assigned to some cellular roles, and 2427 being unannotated (Costanzo *et al*., 2001). A challenging task that lies ahead is to find the functional roles of these unannotated proteins. Several research groups have developed methods for functional annotation. The classical way is to find homologies between a protein and other proteins in protein databases using programs such as FASTA (Pearson, 2000) and PSI-BLAST (Altschul *et al*., 1997), and then predict functions based on sequence homologies. Besides, functional predictions have been modeled as pattern recognition problems based on sequence homologies and structural information (King *et al*., 2001) as well as phenotype data (Clare and King, 2002).

When function cannot be inferred based on sequences similarity, one must rely on true *ab initio* prediction methods. It is a generally accepted paradigm that the function of a protein is determined by its three-dimensional structure, and that the structure is determined by the sequence of the protein. Given this paradigm, it would be logical to think that *ab initio* function prediction could be done by first predicting the structure of the protein, and subsequently predict the function from the structure. However, both steps in this approach are likely to be very difficult to solve.

Knowing the structure of a protein does not mean that it is necessarily possible to figure out what the protein does, even though it is of course a big help (Norin and Sundstrom, 2002). This is because the function of a protein depends on its cellular context. Also, post-translational modifications can profoundly alter the function (and structure) of a protein. Predicting the function of a protein from its structure may therefore very well turn out to be as difficult as the protein folding problem.

However, since proteins collaborate or interact with one another for a common purpose, it is possible to deduce functions of a protein through the functions of its interaction partners (Deng *et al*.,2002; Letovsky and Kasif, 2003). The protein-protein interaction network describes a neighborhood structure among the proteins. If two proteins interact, they are neighbors of each others. For an unannotated protein, the functions of its neighbors can tell us something about the function of the

unannotated protein. For a given function, if most of the neighbors of a protein have the function, it is more likely to be believed that the protein have the same function.

It should be noted that the interaction partners for a protein may belong to different functional categories. It is this complex network of within function and cross-function interactions that makes the problem of functional assignments a difficult task. Methods based on frequencies of interaction partners having certain functions of interest (Schwikowski *et al*., 2000) and on χ2-statistics (Hishigaki *et al*., 2001) have been applied to assign functions to unannotated proteins.

Schwikowski *et al*. (2000) proposed to infer the functions of an unannotated protein based on the frequencies of its neighbors having certain functions. They assign k functions to the unannotated protein with the k largest frequencies in its neighbors. This approach will be referred as the *neighboring counting method*. This approach does not consider the frequency of the proteins having a function among all the proteins. If a function is more common than other functions among all the proteins, the probability that an unannotated protein has this function should be higher than the probability that it has other functions even if the protein does not have interaction partners.

Hishigaki *et al*. (2001) developed another method to infer protein functions based on χ2-statistics. For a protein P_i, let $n_i(j)$ be the number of proteins interacting with P_i and having function F_j . Let $e_i(j) = \#\text{Nei}(i) \times \pi_j$ be the expected number of proteins in Nei(i) having function F_j , where #Nei(i) is the number of proteins in Nei(i). Define

$$S_i(j) = \frac{(n_i(j) - e_i(j))^2}{e_i(j)}. \tag{3.1}$$

For a fixed k, they assign an unannotated protein with k functions having the top k χ2-statistics. Although this approach takes the frequency of the proteins having a function into consideration, $n_i(j)$ is generally small and the applicability of the χ2-statistics is questionable.

Another approach has been developed by Deng *et al*. (2002) in which they apply the theory of Markov random fields to infer a protein's functions using protein-protein interaction data and the functional annotations of its interaction protein partners. For each function of interest and a protein, they predict the probability that the protein has that function using Bayesian approaches. Unlike in other available approaches for protein annotation where a protein has or does not have a function of interest, they give a probability for having the function. This probability indicates how certain it can be believed about the prediction.

Recently, Letovsky and Kasif (2003) applied a method of assigning functions based on a probabilistic analysis of graph neighborhoods in a protein-protein interaction network. The method exploits the fact that graph neighbors are more likely to share functions than nodes which are not neighbors. A binomial model of local neighbor function labeling probability is combined with a Markov random field propagation algorithm to assign function probabilities for proteins in the network. The method has been applied on a protein-protein interaction dataset for the yeast Saccharomyces cerevisiae using the Gene Ontology (GO) terms as function labels. The method reconstructed known GO term assignments with high precision, and produced putative GO assignments to 320 proteins that currently lack GO annotation, which represents about 10% of the unlabeled proteins in *Saccharomyces cerevisiae*.

Part of the reason why it is difficult to relate the chemical function of a protein to its biological purpose is that proteins do not function alone. To understand the function of a protein, it must be considered in its proper cellular context, for example by appreciating how the cell would behave without it (Attwood and Miller, 2001). Many proteins are parts of larger complexes, which are the functional units that fulfill a role in the cell (Gavin *et al*., 2002). In this case it can be argued that all the proteins that form the complex should also have the same function. Since a protein does not perform its function alone but in the context of many other proteins as well as other biomolecules, it is highly relevant to study the interaction partners of a protein in order to understand its function (Eisenberg *et al*., 2000; Ho *et al*., 2002).

The previous approaches to infer the unknown function of a class of proteins have exploited sequence similarities or clustering of co-regulated genes (Harrington *et al*., 2000), phylogenetic profiles (Pellegrini *et al*.,1999), protein-protein interactions (Uetz *et al*., 2000; Ito *et al*., 2000; Schwikowski *et al*.,2000; Deng *et al*., 2002), and protein complexes (Gavin *et al*., 2002; Ho *et al*., 2002). Table 3.1 summarizes different approaches and techniques to infer and predict protein function.

Table 3.1: Computational methods for protein function prediction.

Approach	**Technique**	**Researches**
Sequence alignments	FASTA	Pearson, 2000
	PSI-BLAST	Altschul *et al*., 1997
Multiple sequence alignments	BLOCKS	Henikoff & Henikoff, 1994
	PRINTS	Attwood *et al*., 1997
	PRODOM	Sonnhammer & kahn, 1994
Protein structure prediction	Hidden Markove Models	Karplus *et al*., 1997
	Nearest-neighbor algorithms	Salamov & Solovyev 1995
Phylogenetic patterns	Statistical methods	Pellegrini *et al*., 1999
Gene expression data analysis	SVM	Brown *et al*., 2000
	Statistical algorithm	Eisen *et al*., 1998
Family Identification	Normalized cuts clustering algorithm	Abascal & Valencia, 2003
Protein-protein interaction	n-neighbouring proteins	Hishigaki *et al*., 2001
	Markov random fields and Bayesian networks	Deng *et al*., 2002
	Global optimization and simulated annealing	Vazquez *et al*., 2003
	Markov random fields and label propagation algorithm	Letovsky & Kasif 2003

3.2 Methods to study protein-protein interactions

Protein-protein interactions are working at almost every level of cell function, in the structure of sub-cellular organelles, the transport machinery across the various biological membranes, packaging of chromatin, the network of sub-membrane filaments, muscle contraction, and signal transduction, regulation of gene expression, to name a few (Donaldson *et al.*, 2003) . Abnormal protein-protein interactions have

implications in a number of neurological disorders such as Creutzfeld-Jacob and Alzheimer's disease.

Because of their importance in cell development and disease, protein-protein interactions have gained a lot of attention among researchers for many years. It has emerged from these studies that there is a strategy of mixing and matching of domains that specify particular classes of protein-protein interactions. There are a large number of methods to detect protein-protein interactions. Each of the approaches has its own strengths and weaknesses, especially with regard to the sensitivity and specificity of the method. A high sensitivity means that many of the interactions that occur in reality are detected by the method. A high specificity indicates that most of the interactions detected by the screen are also occurring in reality.

Co-immunoprecipitation is considered to be the gold standard assay for protein-protein interactions, especially when it is performed with endogenous (not overexpressed and not tagged) proteins (Gharakhanian *et al*., 1988). The protein of interest is isolated with a specific antibody. Interaction partners which stick to this protein are subsequently identified by western blotting. Interactions detected by this approach are considered to be real. However, this method can only verify interactions between suspected interaction partners. Thus, it is not a screening approach to identify unknown protein-protein interactions.

The yeast two-hybrid screen investigates the interaction between artificial fusion proteins inside the nucleus of yeast (Bartel and Fields, 1997). This approach can identify binding partners of a protein in an unbiased manner. However, this method suffers from high false-positive rate which makes it necessary to verify the identified interactions by co-immunoprecipitation.

Tandem affinity purification (TAP) detects interactions within the correct cellular environment (e.g. in the cytosol of a mammalian cell) (Rigaut *et al*., 1999). This is a big advantage compared to the yeast two-hybrid approach. However, the TAP tag method requires two successive steps of protein purification. Thus, it can not readily detect transient protein-protein interactions. It is also not efficient to

detect physical protein-protein interactions that exist in different cellular environment. This is especially important when studying the interaction network in the organism's genome which becomes very significant in the post-genomic era.

Quantitative immunoprecipitation combined with knock-down (QUICK) relies on co-immunoprecipitation, quantitative mass spectrometry (SILAC) and RNA interference (RNAi). This method detects interactions among endogenous non-tagged proteins (Selbach and Mann, 2006). Thus, it has the same high confidence as co-immunoprecipitation. However, this method also depends on the availability of suitable antibodies.

Dual Polarisation Interferometry (DPI) is a method that can be used to measure protein-protein interactions. DPI provides real-time, high-resolution measurements of molecular size, density and mass. However this method can not be used to detect new protein-protein interactions.

3.3 Predicting Protein-Protein Interactions

Protein-protein interactions play a crucial role in protein function. Hence, the ability to computationally recognize protein interaction sites and to identify specific interface residues that contribute to the specificity and affinity of protein interactions has important implications in a wide range of clinical and industrial applications.

Until recently, information about protein–protein interactions was gathered via experiments that were individually designed to identify and validate a small number of specifically targeted interactions. This traditional source of information has been augmented recently by the results of high-throughput experiments designed to exhaustively probe all the potential interactions within entire genomes (Table 3.2). However, the many discrepancies between the interacting partners identified in high-throughput studies and those identified in small scale experiments highlight the need for caution when interpreting results from high-throughput studies.

Table 3.2: High-throughput experimental approaches to the determination of protein-protein interactions.

Method	**References**	**Features**
Yeast two-hybrid	Uetz *et al*., 2000	The first comprehensive studies in yeast
	Ito *et al*., 2000	
	Newman *et al*., 2000	
	Boulton *et al*., 2002	Combined analysis of yeast two-hybrid interactions together with phenotype and expression data
	Walhout *et al*., 2002	
Affinity purification/mass	Ho *et al*., 2002	Purification of overexpressed, epitope-tagged proteins in yeast
spectrometric identification	Gavin *et al*., 2002	TAP purification of complexes expressed at physiological levels in yeast
Protein chips	Zhu *et al*., 2001	High-throughput detection of interactions with proteins over-expressed and immobilized on microscope slides to form a proteome microarray
Synthetic lethals	Tong *et al*., 2001	High-throughput identification of synthetic lethal double mutants. Synthetic lethal mutants often correspond to physically interacting protein pairs.
Phage display	Tong *et al*., 2002	Phage display identification of binding motifs followed by computational identification of potential interacting partners and a yeast two-hybrid validation step

High-throughput experimental techniques enable the study of protein-protein interactions at the proteome scale through systematic identification of physical interactions among all proteins in an organism. High-throughput protein-protein interaction data, with ever-increasing volume, are becoming the foundation for new biological discoveries.

A great challenge to bioinformatics is to manage, analyze, and model these data. Comparison between experimental techniques shows that each high-throughput technique such as yeast two-hybrid assay or protein complex identification through mass spectrometry has its limitations in detecting certain types of interactions and they are complementary to each other. Moreover the overlap between these high-throughput experiments is very small as shown in Figure 3.2.

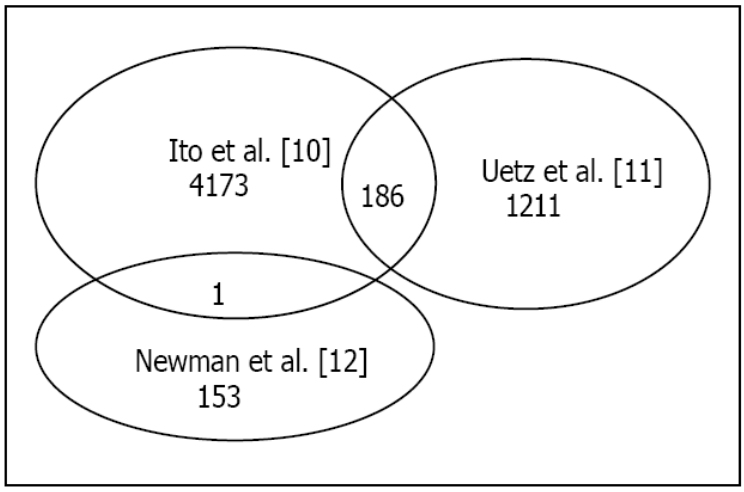


Figure 3.2: The overlap between different high-throughput experiments.

The limitations of the experimental methods to identify protein-protein interactions highlight the need for computational methods to infer and predict protein-protein interactions. As a result, complementary computationally methods capable of accurately predicting interactions would be of considerable value. Furthermore, computational methods for the prediction of protein interactions will provide more data which will enable predicting protein function more precisely since

the function of proteins with three or more partners can be more accurately predicted than with information about one partner.

It is also important to note that computational methods that use protein sequences, domain and structure information to predict protein-protein interaction can expand the scope of experimental data and increase the confidence of certain protein-protein interaction pairs.

Protein-protein interaction data correlate with other types of data, including protein function, subcellular location, and gene expression profile. Highly connected proteins are more likely to be essential based on the analyses of the global architecture of large-scale interaction network in yeast. The use of protein-protein interaction networks, preferably in conjunction with other types of data, allows assignment of cellular functions to novel proteins and derivation of new biological pathways.

Several approaches have been proposed for predicting protein-protein interaction sites from amino acid sequence or from a combination of sequence and structural information (see Table 3.3.). For example, based on their observation that proline residues occur frequently near interaction sites, Kini and Evans (1996) predicted potential protein-protein interaction sites by detecting the presence of "proline brackets." Building on their systematic patch analysis of interaction sites, Jones and Thornton (1997) successfully predicted interfaces in a set of 59 structures using a scoring function based on six parameters. Gallet *et al*. (2000) identified interacting residues using an analysis of sequence hydrophobicity based on a method previously developed by Eisenberg *et al*. (1984) for detecting membrane and surface segments of proteins.

Table 3.3: Computational methods for protein-protein interactions prediction.

Approach	**Technique**	**References**
Identifying interacting sequence motif pairs.	Statistical Method	Wojcik & Schachter, 2001
Co-occurrence of sequence domains.	Probabilistic model	Deng *et al*., 2002
Gene fusion	Rosetta stone	Marcotte *et al*., 2000
Threading-based interaction energy evaluation.	Statistical methods	Lu *et al*., 2002
Phylogenetic profile method.	Statistical methods	Pellegrini *et al*., 1999 Craig and Liao, 2007
Gene Ontology	Semantic similarity search	Wu *et al.*, 2006
Identification of Surface residues	SVM	Ofran & Rost, 2003
		Koike & Takagi, 2003
	Statistical Method	Gallet *et al*., 2000
Primary structure based prediction	SVM	Bock & Gough, 2001
		Dohkan *et al*., 2003
		Ben-Hur & Noble, 2005
		Dohkan *et al*., 2006
	SVM + Attraction-repulsion model	Gomez *et al*., 2003
	Bayesian Networks	Jansen *et al*., 2003 Lin *et al*., 2004
	Set Cover Approach	Huang *et al*., 2007

Prediction of interaction sites in proteins of known structure usually focuses on the location of hydrophobic surface clusters on proteins. In one study, this method predicted the correct interaction site in 25 out of 29 cases (Zhou and Shan, 2001). Other methods include solvation potential, residue interface propensity, hydrophobicity, planarity, protrusion, and accessible surface area. Among a test set of 28 homodimers, the known interface site was found to be amongst the most planar, protruding, and accessible patches, and amongst the patches with highest interface propensity. Nevertheless, one of the algorithms (PATCH) that uses multiple parameters predicted the location of interface sites in known complexes only for 66% of the structures.

Based on the idea that domains mediate the interactions between proteins, Ng *et al*. (2003) collected data from three data sources to develop the database of interacting domains (InterDom). The first one is the experimentally derived protein interaction data from the Database of Interacting Proteins (DIP) (Xenarios *et al*., 2002). The second source is the intermolecular relationship data from protein complexes and the last one is the computationally predicted data from Rosetta Stone sequences. Then they infer putative domain-domain interaction based on the collected data. This is very helpful when inferring protein-protein interactions for proteins partners that have domain structure.

Another approach is to predict protein-protein interactions from genome sequences. Several attempts have been made to achieve that. The major methods are:

- Rosetta stone proteins: Some protein sequences have been found to be split into two independent proteins in other organisms. From such sequences it has been concluded that the two independent proteins form a complex, based on the (covalent) association in the former organism. Such fusion proteins are called Rosetta stone proteins (Edward *et al*., 1999). Supposedly they predict interactions among related proteins. Example: human succinyl CoA transferase is split in E. coli into acetate CoA transferases alfa and beta subunits.

- Phylogenetic profiles: Some protein pairs are evolutionarily maintained together in many different organisms. It has been concluded that such "co-evolving" proteins are associated either functionally or even physically, i.e. by a protein-protein interactions (Marcotte *et al*., 2000). While the latter is not always true, it has been found to be true in a number of cases, such as the yeast proteins Hog1 and Fus3. Also the phylogenetic species tree of the reference genomes can be used as a guide tree for hierarchical clustering of the orthologous proteins (Craig and Liao, 2007). They have shown that the phylogenetic tree can be used as a guide to extract intra-matrix correlations in the distance matrices of orthologous proteins, where these correlations are represented as intermediate distance matrices of the ancestral orthologous proteins.

By measuring the similarity between two Gene Ontology (GO) terms with a relative specificity semantic relation, Wu *et al.*, (2006) proposed a method of reconstructing a yeast protein–protein interaction map that is solely based on the GO annotations. The method was validated using high-quality interaction datasets for its effectiveness. Based on a Z-score analysis, a positive dataset and a negative dataset for protein–protein interactions were derived. This analysis could be efficient on predicting functional protein-protein interactions based on the GO terms similarity. However, it could suffer from high rate of false positive since many protein share similar function but are not physically interacting.

In addition to the above methods for predicting protein-protein interactions from genome sequence, machine learning methods have been recently applied. It is based on the idea that a pattern of interactions can be learned from the available protein-protein interactions data. Using machine learning methods and techniques could be very useful in terms of producing vast amount of possible protein-protein interactions. It could also assist the biologists in pursuing for further analysis on the potential interactions.

Deng *et al*. (2002) proposed a probabilistic prediction model for inferring domain interactions from protein interaction data. The maximum likelihood estimation technique is mainly used in their method. The PFAM database is used to extract domain information and the MIPS database is used to test their model, but they also take single domain pair as a basic unit of protein interactions. The approach taken by Kim *et al*. (2002) shares this assumption with Deng *et al*., but they both suffer from the low sensitivity and specificity of the predictions.

Also looking only at the sequence information of proteins, Goffard *et al*. (2003) developed IPPRED, a web based server for the inference of proteins interactions. IPPRED infers the possibility of the interaction of the two proteins A and B by looking if there is an interacting protein pair C and D which are homologous to A and B (or B and A).

It has been suggested that protein domains mediate protein-protein interactions. Riley *et al*., (2005) describe domain pair exclusion analysis (DPEA), a method for inferring domain interactions from databases of interacting proteins. DPEA features a log odds score, E_{ij}, reflecting confidence that domains *i* and *j* interact. They analyzed 177,233 potential domain interactions underlying 26,032 protein interactions. In total, 3,005 high-confidence domain interactions were inferred, and were evaluated using known domain interactions in the Protein Data Bank. DPEA could be useful in guiding experiment-based discovery of previously unrecognized domain interactions.

However, DPEA detects only the domain interactions best supported by multiple observed protein interactions. Hence, it is expected to suffer from low sensitivity and high specificity in the prediction results. DPEA's sensitivity may be impaired by the high rate of false negatives in existing interaction datasets, particularly in those organisms that have not been probed by high-throughput methods. Indeed, using the defined set of known positive and putative negative domain interactions in the PDB, they obtained a sensitivity of 6%. However, the specificity of 97% in the same test underscores the stringency of the E score. A more informative measure of DPEA's accuracy may be its positive predictive value of

70%, implying that roughly 2/3 of the high-confidence domain interactions inferred by DPEA are true positives; the remaining 1/3 are likely false positives.

Based on the currently available protein-protein interaction and domain data, (Huang *et al*., 2007) described Maximum Specificity Set Cover (MSSC) method for the prediction of protein-protein interactions. In this approach, they mapped the relationship between interactions of proteins and their corresponding domain architectures to a generalized weighted set cover problem. The application of a greedy algorithm provides sets of domain interactions which explain the presence of protein interactions to the largest degree of specificity.

Utilizing domain and protein interaction data of *S. cerevisiae*, MSSC enables prediction of previously unknown protein interactions, links that are well supported by a high tendency of coexpression and functional homogeneity of the corresponding proteins. Focusing on concrete examples, they showed that MSSC reliably predicts protein interactions in well-studied molecular systems, such as the 26S proteasome and RNA polymerase II of S. cerevisiae.

However, MSSC algorithm only allows predictions between proteins with well-known domain structure as well as known interactions among the respective domains. In this approach, other sequence information is not included but only inferring potential domain interactions by counting the occurrence of all possible domain pairs that the domain structure of interacting proteins. This implies a method that risks an elevated level of noise in the determination of potential domain interactions. Accordingly, the error proneness of protein interactions in the respective training sets is another source of potential noise, impacting the quality of predictions.

In addition to these approaches, several different methods that rely on multiple sequence alignment and exploit conserved residues or correlated mutations to detect protein-protein interaction sites have been proposed (Lichtarge *et al*., 1996; Pazos *et al*., 1997). More recently, methods using a support vector machine (SVM) based on primary sequence and associated physicochemical properties have been developed to predict protein-protein interactions (Bock and Gough, 2001; Dohkan *et*

al., 2003). The use of SVM for predicting protein-protein interactions will be discussed in the next section.

3.4 Support Vector Machines

The Support Vector Machine (SVM) is a binary classification algorithm. As such it is well suited for the task of discriminating between interacting and non-interacting protein pairs. SVMs have demonstrated high classification ability in the field of prediction of protein-protein interaction, functional classification of proteins, protein fold recognition, and prediction of subcellular location. SVMs have previously been used in the prediction of protein-protein interaction (Bock and Gough, 2001) (Dohkan *et al*., 2003) (Koike *et al*., 2003) (Chung *et al*., 2004).

The SVM is based on the idea of constructing the maximal margin hyperplane in the feature space (Vapnik, 1995). Suppose we have a set of labeled training data $\{\boldsymbol{x}_i, y_i\}$, $i = 1,\ldots, n$, $y_i \in \{1,-1\}$, $\boldsymbol{x}_i \in \mathrm{R}^d$, and have the separating hyperplane $(\boldsymbol{w} \,.\, \boldsymbol{x}) + b = 0$, where feature vector: $\boldsymbol{x} \in \mathrm{R}^d$, $\boldsymbol{w} \in \mathrm{R}^d$ and $b \in \mathrm{R}$. In the linear separable case the SVM simply looks for the separating hyperplane that maximizes the margin by minimizing $\|\boldsymbol{w}\|^2/2$ subject to the following constraint:

$$y_i(\boldsymbol{w} \,.\, \boldsymbol{x}_i + b) \geq 1 \qquad \forall i \tag{2.2}$$

In the linear non-separable case, the optimal separating hyperplane can be found by introducing slack variables ξ_i, $i = 1,\ldots, n$ and user-adjustable parameter C and then minimizing $\|\boldsymbol{w}\|^2/ 2 + C \Sigma_i \xi_i$, subject to the following constraints:

$$y_i(\boldsymbol{w} \,.\, \boldsymbol{x}_i + b) \geq 1 - \xi_i$$
$$\xi_i \geq 0 \quad \forall\, i = 1,\ldots, n. \tag{2.3}$$

The dual optimization is solved here by introducing the Lagrange multipliers α_i for the non-separable case. Because linear function classes are not sufficient in

many cases, we can substitute $\Phi(x_i)$ for each example x_i and use the kernel function $K(x_i,x_j)$ such that $\Phi(x_i).\Phi(x_j)$. We thus get the following optimization problem:

$$\max_{\alpha} \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{n} \alpha_i \alpha_j y_i y_j K(x_i, x_j) \qquad (2.4)$$

subject to $0 \le \alpha_i \le C, \quad i = 1,..., n.$

and $$\sum_{i=1}^{n} \alpha_i y_i = 0 \qquad (2.5)$$

SVM has the following advantages to process biological data (Bock and Gough, 2001): (1) SVM is computationally efficient and it is characterized by fast training which is essential for high-throughput screening of large protein datasets. (2) SVM is readily adaptable to new data, allowing for continuous model updates in parallel with the continuing growth of biological databases. (3) SVM provides a principled means to estimate generalization performance via an analytic upper bound on the generalization error. This means that a confidence level may be assigned to the prediction, and avoids problems with overfitting inherent in neural network function approximation.

Recently, SVM has been used to predict protein-protein interactions. For example, Bock and Gough (2001) used SVM and physicochemical properties of residues such as hydrophobicity and surface tension to predict protein-protein interactions. The prediction methodology reported in their paper generates a binary decision about potential protein–protein interactions, based only on primary structure and associated physicochemical properties. Their results suggest the possibility of proceeding directly from the automated identification of a cell's gene products to inference of the protein interaction pairs, facilitating protein function and cellular signaling pathway identification. As they mention in their paper, their research represents only an initial step in the automated prediction of protein interactions. With experimental validation, further development along these lines may produce a robust computational screening technique that narrows the range of putative

candidate proteins to those exceeding a prescribed threshold probability of interaction.

In a recent paper, Dohkan *et al*. (2003) have proposed a new method to predict protein-protein interactions using Support Vector Machines. In their method, multiple domain effects and the physicochemical features can be considered all at once. They mentioned that the prediction accuracy was clearly improved by adding protein features such as amino acid composition and/or localization. Furthermore, consideration of combined features domain, amino acid composition, and subcellular localization resulted in the best prediction performance: an F-measure of 79%. The present predictions seem to be more accurate than those reported previously.

Moreover they applied their method to the unknown protein pairs, and fount that high scoring protein pairs were likely to have similar GO annotations. These results indicate that the present method is useful inferring likely interactions between unknown protein pairs and/or detect reliable protein interaction from high-throughput data. Further, the similar GO annotation tendencies indicate the possibility of that the biological function may be predicted by the prediction of protein interaction pairs. They also evaluated the effects of using only the physicochemical features on the prediction of protein-protein interactions. They retrained the SVM without using domain information. The results imply that only the use of these physicochemical properties is not sufficient to predict interactions accurately, and domain information is quite informative.

3.5 Bayesian Networks

A Bayesian Network (BN) is a graphical model that encodes probabilistic relationships among variables of interest. When used in conjunction with statistical techniques, the graphical model has several advantages for data analysis (Jensen, 1996). One, because the model encodes dependencies among all variables, it readily handles situations where some data entries are missing. Two, a Bayesian network can be used to learn causal relationships, and hence can be used to gain understanding

about a problem domain and to predict the consequences of intervention. Three, because the model has both a causal and probabilistic semantics, it is an ideal representation for combining prior knowledge (which often comes in causal form) and data. Four, Bayesian statistical methods in conjunction with Bayesian networks offer an efficient and principled approach for avoiding the over fitting of data.

Given a set of variables $D = \{X_1, X_2 \ldots X_N\}$, where each variable X_i could take values from a set Val(X_i), a Bayesian Network describes the probability distribution over this set of variables. The capital letters as X,Y are used to denote variables and lower case letters as x,y to denote values taken by these variables. Formally, a BN is an annotated directed acyclic graph (DAG) that encodes a joint probability distribution. A network B can be denoted as a pair $B = \langle G, \Theta \rangle$, where G is a DAG whose nodes symbolize the variables of D, and Θ refers to the set of parameters that quantifies the network. G embeds the following conditional independence assumption:

Each variable X_i is independent of its non-descendants given its parents.

Θ includes information about the probability distribution of a value x_i of a variable X_i, given the values of its immediate predecessors. The unique joint probability distribution over $\{X_1, X_2 \ldots X_N\}$ that a network B describes can be computed using:

$$P_B(X_1 \ldots X_N) = \prod_{i=1}^{N} P(x_i \mid parents(X_i)) \qquad (2.6)$$

Since large-scale data sets of protein interactions can be very noisy and can lead to inaccuracies when trying to identify protein interactions on a genome-wide scale and the data in the literature can be incomplete and contradictory, the Bayesian Networks can be considered as a candidate approach to cope with this problem.

Jansen *et al*. (2003) have developed a Bayesian approach for integrating weakly predictive genomic features into reliable predictions of protein-protein interactions. They constructed protein interaction network for the yeast genome. In

this network, they combined four large-scale, highthroughput data sets of protein interactions from the literature. This network is known as probabilistic interactome experimental (PIE).

PIE is simply taking the existing but noisy interaction data sets and trying to integrate them to create an optimal experimental interactome. In constructing the network, each source of information is assessed by comparing it against a set of "gold standards" of known positive and negative protein interactions. The positives are taken from the Munich Information Center for Protein Sequences catalog of known protein complexes. The negative protein interactions include proteins that are known to be separated in different subcellular compartments. When the network is compared to the gold standards, the predicted network turns out to be more accurate than the existing experimental data sets. One of the main achievements of their research is to predict protein interactions to a well-defined level of accuracy from non-interaction information and show that these predictions are essentially as accurate, if not more accurate, as directly getting the highthroughput interaction data.

Another research group has conducted an assessment study based on the genomic features used in a Bayesian network, random forest and logistic regression to predict protein-protein interactions genome-wide in yeast (Lin *et al*., 2004). The non-Bayesian methods do not require prior information needed for the Bayesian approach, and can fully utilize the raw data without discretization. They reported that the logistic model performs similarly as the Bayesian method in terms of classifications and, like the Bayesian method, produces estimated probabilities that two proteins interact. As a dichotomous classifier, the random forest method outperforms the other methods considered and efficiently uses the information, although it is computationally more expensive. In particular, its importance measure provides a more objective assessment of different genomic features on predicting protein-protein interactions than simply considering contributions to model fitting.

3.6 Summary

This chapter has discussed the literature in the field of protein function prediction problem. It has showed that it is possible to infer and protein function from protein-protein interactions data. Many researchers have used the available experimental data of protein-protein interactions to infer and predict protein function of the unannotated proteins. However, according to the literature, the experimental data is suffering from many false positive and has many discrepancies between different experiments results. In the meantime there are many research have been proposed to predict the protein-protein interaction using computational methods from protein primary structure and associated features. Therefore, it would possible to combine and validate the computationally predicted and the experimental protein interactions data to construct more reliable dataset for predicting proteins function. Hence, this research focuses on predicting protein-protein interactions from protein sequence information using the support vector machines and Bayesian approach.

CHAPTER 4

THE RESEARCH METHODOLOGY

Based on the literature review discussed in Chapter 3, the main requirement is to develop a machine learning technique that is capable to infer and predict protein-protein interactions data. Basically, this chapter describes the research methodology needed to fulfill the research objective. The datasets that is used in the experiments of this research is presented and discussed as well as the evaluation measures of the system performance are also discussed. In addition to the methodology, the expected outcome at each stage of the investigation will be presented as well. At the end of this chapter the assumptions and the limitations of this research will be presented.

4.1 Research Design

This research is an applied, scientific research using the problem oriented approach. It involves several important issues: protein-protein interactions, Support Vector Machines and Bayesian Methods. In this work, a machine learning method based on a support vector machine (SVM) combined with a kernel function was developed for the prediction of protein-protein interactions based only on the primary sequences of proteins. The general research framework is presented in the next section.

4.2 General Research Framework

Applying the conventional methods of machine learning approaches including support vector machines without augmentation, to biological data bases does not achieve good performance. This is due to the nature of the biological data which is dynamic rather than static data conventionally used in patter recognition problem solving. In this research, examining and studying different protein sequence feature is carried out in order to identify the best feature that can be used to accurately predict protein-protein interactions from protein sequences information. Including biological information represented in the feature selection is essential for successful machine learning approach.

Hence, this research framework is initiated by studying and comparing different sequence features for the prediction of protein-protein interactions using the support vector machines. Positive and negative datasets are required for training the support vector machines. Although, constructing a positive dataset (i.e. pairs of interacting proteins) is relatively an easy task by using one of the available databases of interacting proteins, there is no data on experimentally confirmed non-interacting protein pairs have been made available. To cope with this problem, some researchers created an artificial negative protein interaction dataset for *Saccharomyces cerevisiae* by randomly generating protein pairs from this organism that are not described as interacting in the Database of Interacting Proteins (DIP) without putting any further restrictions on such pairs, as in (Deane *et al*., 2002; Chung *et al*., 2004).

One problem with this approach is that in many cases selected non-interacting protein pairs will possess features that are substantially different from those typically found in the positive interaction set. This effect may simplify the learning task and artificially raise classification accuracy for training data. There is no guarantee, however, that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify. However, this approach could be used for comparing different features or algorithms since the error will be uniform among all features or algorithms.

Due to the unavailability of experimentally confirmed non-interacting protein pairs, the problem of predicting protein-protein interactions is essentially one-class classification problem. Accordingly, the One-Class SVM is proposed in this research framework. Different kernels with different parameters are to be studied and compared.

In the mean time, several high-throughput experimental methods have been developed in efforts to map the interactions among all of the proteins encoded by a genome. While the data from these studies has been useful to biologist, it also has several shortcomings. In particular, the results from high-throughput interaction mappings have low accuracy and suffer from high false positive rate. Estimated error rates of high-throughput interaction results range from 41 to 90% (Deane *et al*., 2001; von Mering *et al*., 2002). Therefore, designing and implementing knowledge-based kernel that incorporate the probabilistic biological information could improve the performance of the SVM. In this research framework, a Bayesian kernel is proposed.

The general framework for this work is presented in Figure 4.1. The general research framework can be divided into four main phases as following:

Phase 1: The development of domain model where the current and previous research will be reviewed in order to identify protein sequence features for protein-protein interactions prediction.

Phase 2: The development of support vector machines to predict protein-protein interactions from protein sequence information using different features.

Phase 3: The development of the One-Class SVM to predict protein-protein interactions using only positive data for training phase.

Phase 4: The development of a Bayesian kernel for SVM to predict protein-protein interactions by incorporating probabilistic information of the existing interactions. It also provides probabilistic output as the likelihood of the interaction.

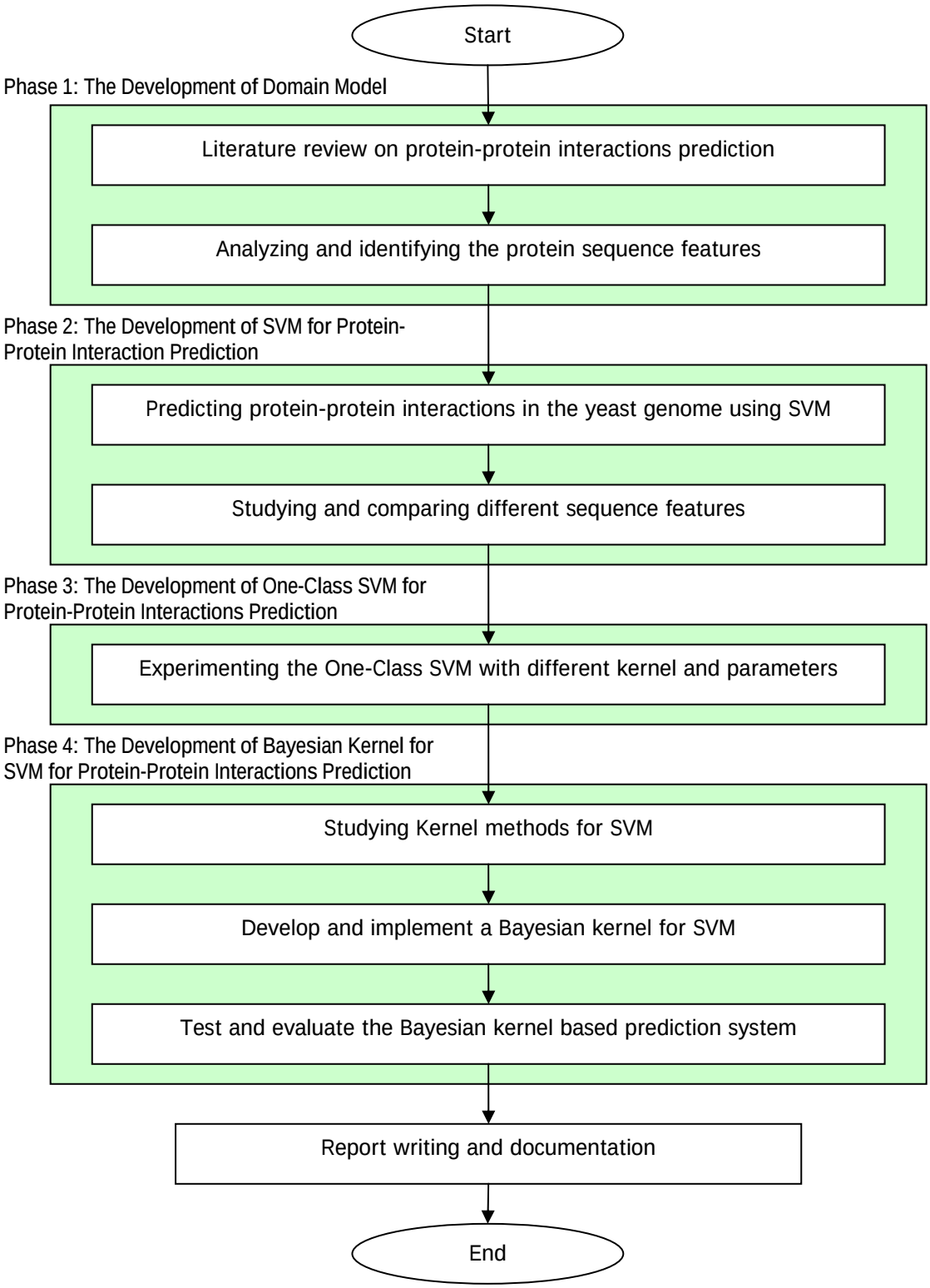


Figure 4.1: The general research operational framework.

The motivation behind using Bayesian approach is that they readily accommodate missing data and they naturally weight each information source according its reliability (Jansen *et al*., 2003). This will be useful for protein interaction data because the overlapped data between different experiments are relatively small (Table 4.1).

Table 4.1: The protein interactions of yeast *Saccharomyces cerevisiae* identified by wet lab experiments.

Proteins	Interactions	Number of Experiments	Number of Interactions
4749	15675	1	13653
		2	1278
		3	407
		4	167
		5	84
		6+	101

The feature vector for each protein will be constructed by representing the domain structure and the physicochemical features of the protein. An interaction between two proteins will represented by the concatenation of these feature vectors of each protein, and was then labeled with +1 for positives and -1 for negatives. Further details on feature vector representation will be discussed in Chapter 5.

4.3 Protein Data Sets

Physical interactions between two proteins of *Saccharomyces cerevisiae* have been obtained from the Database of Interacting Protein (DIP) (Xenarios *et al*., 2002). Among them, interactions identified by only high-throughput methods (defined here as ones that resulted in more than 100 interactions being reported in a single article,

as is described in Deane *et al*., 2001) will be only considered if they appear in the DIP core dataset. The DIP core dataset contains 2609 yeast proteins that are involved in 6355 interactions which have been found with more than one different experimental method. This procedure will yield 4178 interactions as candidate of positive training sets. Negative data will be generated by compiling all possible protein pairs that were not recognized as positive (including high-throughput results) in the DIP databases. Also all protein pairs that are part of a complex comprising more than two proteins will be removed from negative sets, since those pairs have the possibility interacting physically each other.

The number of positive protein pairs is quite small compared to that of potentially negative pairs. The excessive potentially negative examples in the training set lead to yield many false negatives because many of the positive examples are ambiguously discriminative from the negative examples in the feature space. On the other hand, the insufficient negative examples yield many false positives and lead to the fluctuation in the prediction performance, since the number of the training samples becomes small.

The Database of Interacting Proteins (DIP) was initially developed to store and organize information on binary protein-protein interactions that was retrieved from individual research articles (Xenarios *et al*., 2002). Over the course of the last 4 years the progress in genome-scale experimental methods has resulted in rapid identification of binary protein-protein interactions (Ito *et al*., 2000) (Uetz *et al*., 2000) and multi-protein complexes (Gavin *et al*., 2002). On one hand, it prompted enhancements to the database schema that allow the capture, with increased level of detail, of information on the molecular interactions. On the other hand, questions about the reliability of the experiments conducted on a genome-wide scale stimulated development of data quality assessment methods (Deane *et al*., 2001).

The DIP database is implemented as a relational database using an open source PostgreSQL database management system (http://www.postgresql.org). The simplified version of the current database schema is shown in Figure 4.2. The key tables - PROTEIN, SOURCE and EVIDENCE - store, respectively, information on individual proteins, sources of experimental information and information on

individual experiments. The information on protein-protein interactions is stored in two tables - INTERACTION and INT_PRT. Such arrangement of the tables enables description of binary interactions (two entries in the INT_PRT table for each INTERACTION entry) but also of multi-protein complexes (more than two entries in INT_PRT for each INTERACTION entry).

The METHOD table provides a list of controlled vocabulary terms, together with references to the corresponding PSI ontology entries (Hermjakob *et al*., 2004), which are used to annotate the experiments. When available, information on the details of the topology of a molecular complex that was inferred from each experiment is stored in the TOPOLOGY and LOCATION tables. The LOCATION table describes regions of proteins participating in interactions whereas the TOPOLOGY table pairs them into records that describe observed binary interactions. It also specifies the type of interaction inferred from each experiment as one of aggregate (both partners shown to be present in the same complex but not necessarily in direct contact), contact or covalent bond.

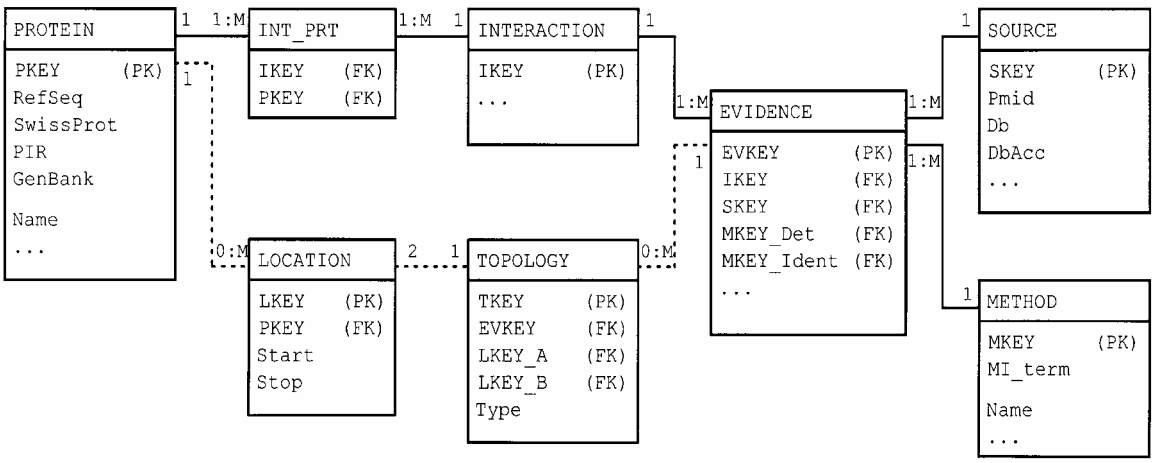


Figure 4.2: A simplified entity-relationship diagram of the DIP database (Xenarios *et al*., 2002).

4.4 Evaluation Measures of the System Performance

The performance of the protein-protein interactions system is measured by how well the system can accurately predict protein-protein interactions using only sequence information. A system can make errors by identifying protein pairs as interacting pairs while they are known to be non-interacting or identifying protein pairs as non-interacting while they are known to be interacting. For a binary classification problem there are two classes {+1,−1}= {interacting, non-interaction}. In order to analyze evaluation measures in family classification, we first explain the contingency table (Table 4.2).

Table 4.2: The contingency table.

	Interacting Pairs	**Non-interacting Pairs**
Predicted Interacting	*True Positive (TP)*	*False Positive (FP)*
Predicted Non-interacting	*True Negative (FN)*	*True Negative (TN)*

The entries of the four cells of the contingency table and a number *n* are described as follows:

TP = number of interacting pairs predicted interacting
FP = number of non-interacting pairs predicted interacting
TN = number of non-interacting pairs predicted non-interacting
FN = number of interacting pairs predicted non interacting
n = TP + FP + TN + FN = Total number of protein pairs

The information encoded in the contingency table is enough to calculate not only the protein-protein interactions prediction evaluation measures, but also the evaluation measures for general classification problems. Accordingly, the general and widely used evaluation measures for classification problem such as sensitivity, specificity, precision, false positive rate (FPR) and receiver operating characteristic

(ROC) can be applied in this research to evaluate the performance of the prediction system. These measures can be defines as follows.

Sensitivity (Recall) is the probability that the classifier result is positive (interacting protein pairs) when the protein pairs are interacting. It can be calculated as following:

$$Sensitivity = Recall = \frac{TP}{TP + FN} \tag{4.1}$$

Specificity is the probability that the classifier result is negative (non-interacting protein pairs) when the protein pairs are non-interacting. It can be calculated as following:

$$Specificity = \frac{TN}{TN + FP} \tag{4.2}$$

Precision is the probability that the protein pairs are interacting when the classifier result is positive (interacting protein pairs). It can be calculated as following:

$$Precision = \frac{TP}{TP + FP} \tag{4.3}$$

False-positive rate is the probability that the classifier result is positive when the protein pairs are non-interacting. It can be calculated as following:

$$FPR = \frac{FP}{TN + FP} = 1 - Specificity \tag{4.4}$$

The ROC is a plot of the true positive rate (sensitivity) against the false positive rate (1-specificity) for a binary classifier system as its discrimination threshold is varied.

4.5 Summary

This chapter explains a detailed framework of the methodology adopted in this research in an attempt to accurately predict protein-protein interactions from protein sequence information. It is assumed in this research that the yeast proteins sequences and protein interactions data are available publicly via the internet as reported in the literature. However, in the following chapters, we are going to present more details methods for feature representation of the protein sequence, followed by details description of the SVM, One-Class SVM and Bayesian kernel.

CHAPTER 5

COMPARISON OF PROTEIN SEQUENC FEATURES FOR THE PREDICTION OF PROTEIN-PROTEIN INTERACTIONS USING SUPPORT VECTOR MACHINES

This chapter describes and discusses the method and implementation used to study and compare protein sequence features for predicting protein-protein interactions using support vector machines. These features are protein domain structures and hydrophobicity. The framework of these experiments and the data preparation is also discussed in this chapter. At the end of this chapter, the results of studying and comparing these features are presented

5.1 Related Work

Over the past few years, several computational approaches to predict protein-protein interaction have been proposed. Some of the earliest techniques were based on the similarity of expression profiles to predict interacting proteins (Marcotte *et al*., 1999), coordinatation of occurrence of gene products in genomes, description of similarity of phylogenetic profiles (Pellegrini *et al*., 1999) or trees (Pazos and Valencia, 2001), and studying the patterns of domain fusion (Enright *et al*., 1999). However, it has been noted that these methods predict protein–protein interactions in a general sense, meaning joint involvement in a certain biological process, and not necessarily actual physical interaction (Eisenberg *et al*., 2000).

Another recent method has been introduced based on the assumption that protein–protein interactions are evolutionary conserved. It involves the use of high-quality protein interaction map with interaction domain information as input to predict an interaction map in another organism (Wojcik and Schachter, 2001).

These methods which are based on genomic information are not universal because the accuracy and reliability of these methods depend on information of protein homology or interaction marks of the protein partners.

For instance, computational methods such as phylogenetic profiles, predict protein-protein interactions by accounting for the pattern of the presence or absence of a given gene in a set of genomes (Marcotte *et al*., 2000; Craig and Liao, 2007). The main limitation of these approaches is that they can be applied only to completely sequenced genomes, which is the precondition to rule out the absence of a given gene. Similarly, they cannot be used with the essential proteins that are common to most organisms (Shen *et al*., 2007). The prediction of functional relationships between two proteins according to their corresponding adjacency of genes is another popular approach. This method is directly applicable only to bacteria, in which the genome order is relatively more relevant (Wojcik and Schachter, 2001).

Consequently, predicting protein-protein interactions based only on protein sequence features has a significant importance for computational methods. The advantage of such a method is that it is much more universal. This is can be done by developing computation methods that predict protein-protein interactions by associating experimental data on interacting proteins with annotated features of protein sequences using machine learning approaches, such as support vector machines (SVM) (Bock and Gough, 2001; Chung *et al*., 2004) and data mining techniques, such as association rule mining (Oyama *et al*., 2002).

The most common sequence feature used for this purpose is the protein domains structure. The motivation for this choice is that molecular interactions are typically mediated by a great variety of interacting domains (Pawson and Nash,

2003). It is thus logical to assume that the patterns of domain occurrence in interacting proteins provide useful information for training PPI prediction methods.

In a recent study, the notion of potentially interacting domain pair (PID) was introduced to describe domain pairs that occur in interacting proteins more frequently than would be expected by chance (Kim *et al*., 2002). Assuming that each protein in the training set may contain different combinations of multiple domains, the tendency of two proteins to interact is then calculated as a sum over log odd ratios over all possible domain pairs in the interacting proteins. Using cross-validation, the authors demonstrated 50% sensitivity and 98% specificity in reconstructing the training data set. In a similar approach, (Ng *et al*., 2003) developed a scoring scheme which takes into account both experimental protein-protein interactions data and interaction pairs derived computationally based on domain fusion analysis.

Different approach based on domain-domain interactions information has been presented in (Gomez *et al*., 2003). They developed a probabilistic model to predict protein interactions in the context of regulatory networks. A biological network is represented as a directed graph with proteins as vertices and interactions as edges. A probability is assigned to every edge and non-edge, where the probability for each edge depends on how domains in two corresponding proteins “attract” and “repel” each other. The regulatory network is predicted as the one with the largest probability for its network topology. Using the Database of Interacting Proteins (DIP) (Xenarios *et al*., 2002), as the standard of truth and the Protein Families Database (PFAM) domains as sequence features, the authors built a probabilistic network of yeast interactions and reported an ROC score of 0.818.

Another sequence feature that has been used to computationally predict protein-protein interactions is the hydrophobicity properties of the amino acid residues. Chung *et al*., (2004) used SVM learning system to recognize and predict protein-protein interactions in the yeast *Saccharomyces cerevisiae*. They selected only the hydrophobicity properties as sequence feature to represent the amino acid sequence of interacting proteins. They reported 94% accuracy, 99% precision, and 90% recall in average.

Although they achieved better results than the previous work using only hydrophobicity feature, their method of generating a negative dataset (i.e. non-interacting proteins pairs) is different from the previous work. They constructed the negative interaction set by replacing each value of the concatenated amino acid sequence with a random feature value. As they mention in their conclusion, this approach simplify the learning task and artificially raise classification accuracy for training data. However, there is no guarantee that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify.

Therefore, in this research we proposed a better and more realistic method to construct the negative interaction set. Then we compared the use of domain structure and hydrophobicity properties as the protein features for the learning system. The choice of these two features is motivated by the above discussed literature.

5.2 Comparison Experiment Framework

In order to compare two protein sequence features for the prediction of protein-protein interactions, we applied the same process on both features, as shown in Figure 5.1. This process starts by generating a dataset of interacting and non-interacting proteins pairs. For the interacting pair, it is simply obtained from the Database of Interacting Protein (DIP). But, there is no dataset of experimentally identified non-interacting proteins. Therefore we use a random method to generate proteins pairs, and then delete all pairs that appear in the DIP. This is acceptable for the purposes of comparing the feature representation since the resulting inaccuracy will be approximately uniform with respect to each feature representation. The Support Vector Machines have been used as the learning system. It has been trained to distinguish between interacting and non-interacting protein pairs using domain and hydrophobicity training sets. The following sections give some details about the methods that were used in this work.

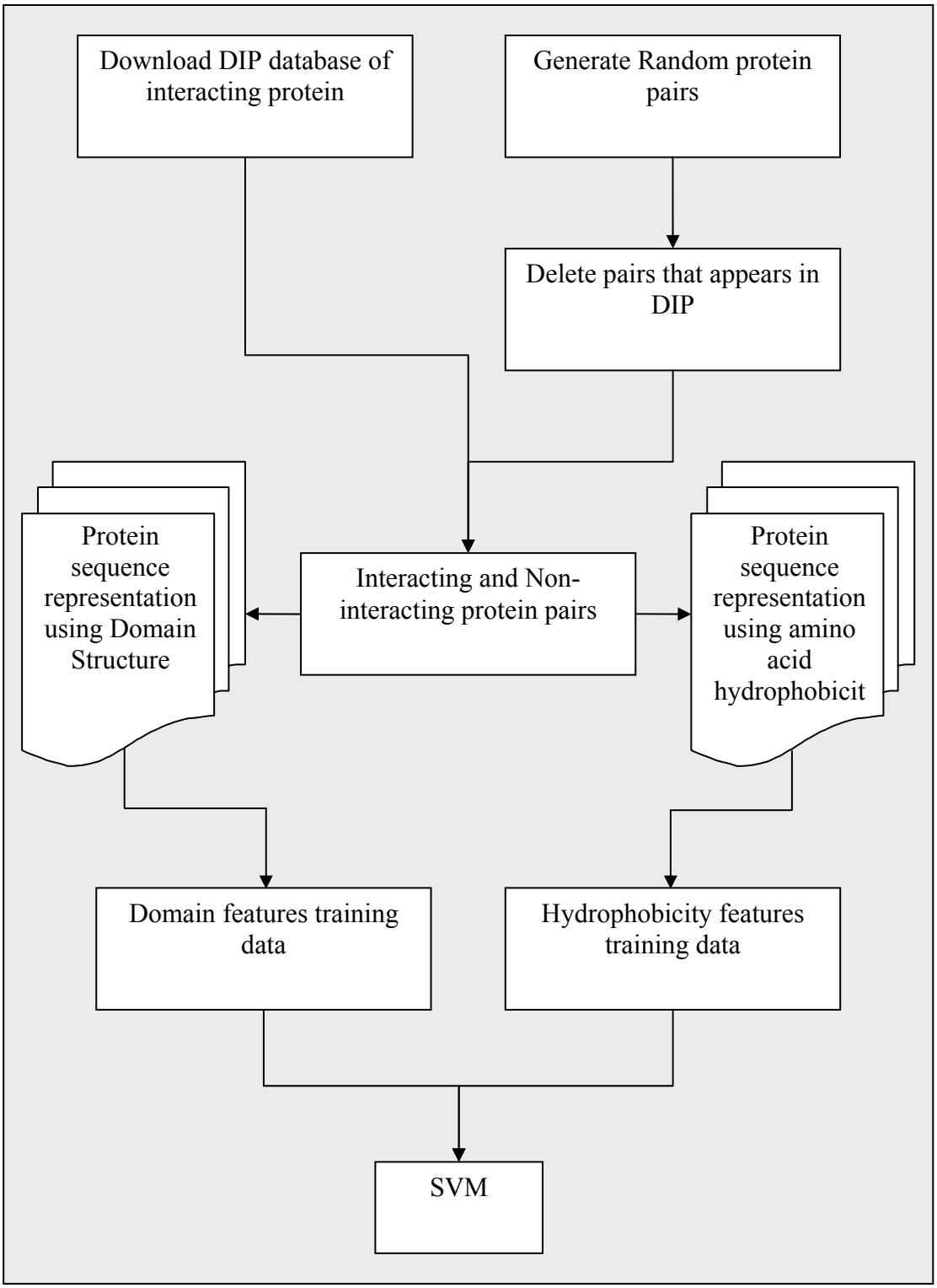


Figure 5.1: The framework of comparing protein sequence features.

5.3 The Support Vector Machines

The support vector machine (SVM) is a binary classification algorithm. Thus, it is well suited for the task of discriminating between interacting and non-interacting protein pairs. The support vector machine was proposed by Boser *et al*., (1992). A detailed analysis of SVMs can be found in (Vapnik, 1995; Cristianini and Shawe-Taylor, 2000; Schölkopf and Smola, 2002). The SVM is based on the idea of constructing the maximal margin hyperplane in the feature space. This unique hyperplane separates the data into two categories $\{-1,+1\}$ with maximum margin (hard margin). It is also known as the optimal hyperplane. Figure 5.2 shows a maximal margin hyperplane, where w is perpendicular to the hyperplane and b is the distance of the hyperplane from the origin. A better generalization capability is expected from (b). A maximal margin hyperplane is given by:

$$\langle w \cdot x \rangle + b = 0 \tag{5.1}$$

corresponding to decision function:

$$f(x) = \text{sgn}(\langle w \cdot x \rangle + b) \tag{5.2}$$

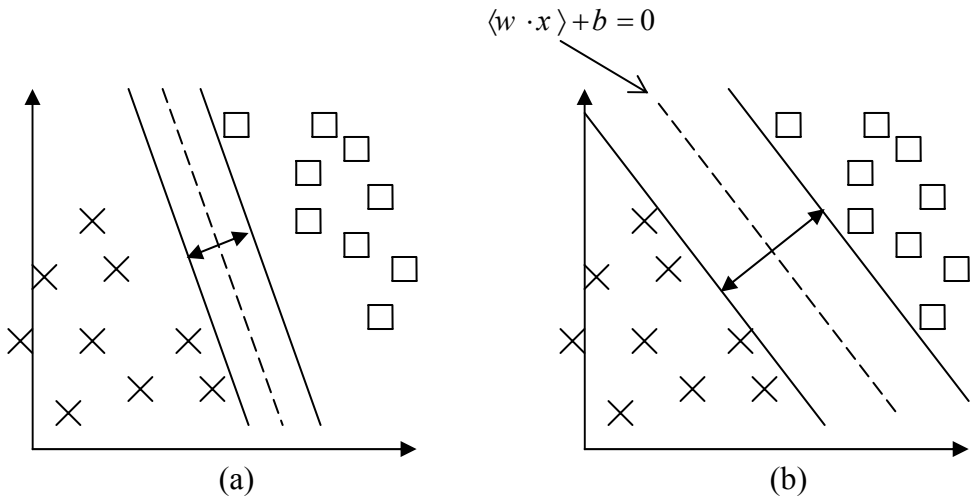


Figure 5.2: (a) A separating hyperplane with small margin. (b)A separating hyperplane with larger margin.

To find a maximal margin hyperplane, one has to solve a Convex Quadratic Optimization Problem (CQOP). CQOP is usually decomposed to reduce the training cost (Osuna *et al*., 1997; Hsu and Lin, 2002).

Given a set of linearly separable instances $\{(x_1, y_1),(x_2, y_2),\ldots,(x_n, y_n)\}$, Figure 5.2 illustrates a binary classification problem. Provided that the problem being separable, there exists a weight vector *w* and *a* threshold *b* such that $y_i \cdot (\langle w \cdot x \rangle + b) > 0,\ (i = 1,\ldots,n)$. Note that the margin measured perpendicularly to the hyperplane, equals $\frac{2}{\|w\|}$. This can be seen by considering two points x_1 and x_2 on the opposite side of the margin.

The value of the margin can be derived as follows:

$$\langle w \cdot x_1 \rangle + b = +1 \tag{5.3}$$

$$\langle w \cdot x_2 \rangle + b = -1 \tag{5.4}$$

$$x_1 = x_2 + \lambda w\ ,\ \text{for any } \lambda \tag{5.5}$$

From Equation (5.3)

$$\begin{aligned} &(w \cdot (x_2 + \lambda w)) + b = +1 \\ &\Rightarrow (w \cdot x_2) + \lambda w \cdot w + b = 1 \\ &\Rightarrow (w \cdot x_2) + b + \lambda w \cdot w = 1 \\ &\Rightarrow \lambda = \frac{2}{w \cdot w} \end{aligned} \tag{5.6}$$

The margin can be calculated as:

$$Mar = |x_1 - x_2| \tag{5.7}$$

From Equations (5.6) and (5.7), we get:

$$Mar = \left|(x_2 + \lambda w) - x_2\right|$$
$$\Rightarrow Mar = \left|\lambda w\right| = \lambda\sqrt{w \cdot w}$$
$$\Rightarrow Mar = \frac{2}{w \cdot w}\sqrt{w \cdot w} = \frac{2}{\|w\|} \tag{5.8}$$

To construct this optimal hyperplane (Figure 5.2), one has to solve the following optimization problem:

$$Minimize \quad \frac{1}{2}\langle w \cdot w \rangle \tag{5.9}$$

Subject to:

$$\langle w \cdot x_i \rangle + b \geq +1 \quad if \quad y_i = +1,$$
$$\langle w \cdot x_i \rangle + b \leq -1 \quad if \quad y_i = -1,$$
$$for\ all\ i = 1, \ldots, n.$$

Putting the above two constraints together, we can write

$$y_i\left(\langle w \cdot x_i \rangle + b\right) \geq 1 \qquad for \ \ i = 1, 2, \ldots n. \tag{5.10}$$

To obtain a solution, we need to introduce the Lagrangian L and Lagrange multipliers α_i.

$$L(w, b, \alpha) = \frac{1}{2}\langle w \cdot w \rangle - \sum_{i=1}^{n} \alpha_i \left(y_i \cdot \left(\langle w \cdot x_i \rangle + b\right) - 1\right) \tag{5.11}$$

Minimization of a convex quadratic optimization problem is equivalent to maximization of its dual. The dual of the above equation is found by taking the

derivate of Lagrangian *L* with respect to the primal variables *w* and *b*. At the solution point (saddle point) these derivatives vanish. Saddle point is defined as a fixed point for which the stability matrix has eigenvalue. The eigenvalue can be defined as follows:

Let Ą be a n × n matrix of real or complex numbers. A real or complex number λ is an eigenvalue of X if and only if, for some nonzero n ×1 matrix Ė

$$Ą\,Ė = \lambda\,Ė \tag{5.12}$$

Now,

$$\frac{\partial L(w,b,\alpha)}{\partial w} = w - \sum_{i=1}^{n} y_i \alpha_i x_i = 0, \tag{5.13}$$

$$\frac{\partial L(w,b,\alpha)}{\partial b} = \sum_{i=1}^{n} y_i \alpha_i = 0, \tag{5.14}$$

Before substituting these relations into the Lagrangian *L*, we will analyze them in detail. The following conditions must be satisfied

$$\sum_{i=1}^{n} y_i \alpha_i = 0, \quad and \quad \alpha_i \geq 0 \quad for\ i = 1,\ldots,n \tag{5.15}$$

The solution vector *w* is given by

$$w = \sum_{i=1}^{n} \alpha_i y_i x_i \tag{5.16}$$

It is clear that the weight vector is a linear combination of the training instances. Karush-Kuhn-Tucker (Kuhn and Tucker, 1951) conditions state that the solution must satisfy the following:

$$\alpha_i \left(y_i \cdot \left(\langle w \cdot x_i \rangle + b \right) - 1 \right) = 0 \tag{5.17}$$

The instances for which $\alpha_i \neq 0$ are known as support vectors and have margin 1. The removal of all other instances does not affect the solution. In other words, α_i is a measure of the importance of an instance for the solution.

By substituting Equations (5.11), (5.13), and (5.16) into the Lagrangian L , we obtain

$$\begin{aligned} L(\alpha) &= \frac{1}{2}\|w\|^2 - \sum_{i=1}^{n} \alpha_i \left(y_i \cdot \left(\langle w \cdot x_i \rangle + b \right) - 1 \right) \\ &= \frac{1}{2}\|w\|^2 - \sum_{i=1}^{n} \alpha_i \left(y_i \cdot \langle w \cdot x_i \rangle + y_i b - 1 \right) \\ &= \frac{1}{2}\|w\|^2 - \sum_{i=1}^{n} \alpha_i y_i \cdot \langle w \cdot x_i \rangle + \sum_{i=1}^{n} \alpha_i y_i b - \sum_{i=1}^{n} \alpha_i \\ &= A - B + C - D \end{aligned} \tag{5.18}$$

From Equations (5.13) and (5.14), $B = 0$ and $C = 0$. Simplifying, we have,

$$L(\alpha) = \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{n} \alpha_i \alpha_j y_i y_j \langle x_i \cdot x_j \rangle \tag{5.19}$$

In other words, the problem that must be solved is

$$\textit{Maximize} \qquad \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{n} \alpha_i \alpha_j y_i y_j \langle x_i \cdot x_j \rangle \tag{5.20}$$

$$\textit{Subject to}: \qquad \alpha_i \geq 0, \quad i = 1, \ldots, n$$

$$\sum_{i=1}^{n} \alpha_i y_i = 0$$

The equivalent kernel version is as follows:

$$Maximize \qquad \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{n} \alpha_i \alpha_j y_i y_j k \left\langle x_i \cdot x_j \right\rangle \tag{5.21}$$

$$Subject\ to: \qquad \alpha_i \geq 0, \quad i = 1, \ldots, n$$

$$\sum_{i=1}^{n} \alpha_i y_i = 0$$

By replacing the value of *w* from Equation (5.16), we can write the classification function in Equation (5.2) as

$$f(x) = \operatorname{sgn}\left(\sum_{i=1}^{n} \alpha_i y_i \left\langle x_i \cdot x \right\rangle + b \right) \tag{5.22}$$

The equivalent kernel version is given by

$$f(x) = \operatorname{sgn}\left(\sum_{i=1}^{n} \alpha_i y_i k \left\langle x_i \cdot x \right\rangle + b \right) \tag{5.23}$$

The only unknown value here is b which can be calculated using

$$\left(y_i \left(\left\langle w \cdot x_i \right\rangle + b \right) - 1 \right) = 0, \quad for\ some\ i\ with\ \alpha_i \neq 0. \tag{5.24}$$

5.3.1 Soft Margin Optimal Hyperplane

The maximal margin hyperplane fails to generalize well when there is a high level of noise in the data. The presence of noise is not uncommon in real world classification problems. The maximal margin hyperplane may be hard to achieve in

the presence of noise. The constraints $y_i\left(\langle w \cdot x_i \rangle + b\right) \geq 1,$ become too restrictive. Figure 5.3 shows the hard margin and soft margin solution when the data contains noise.

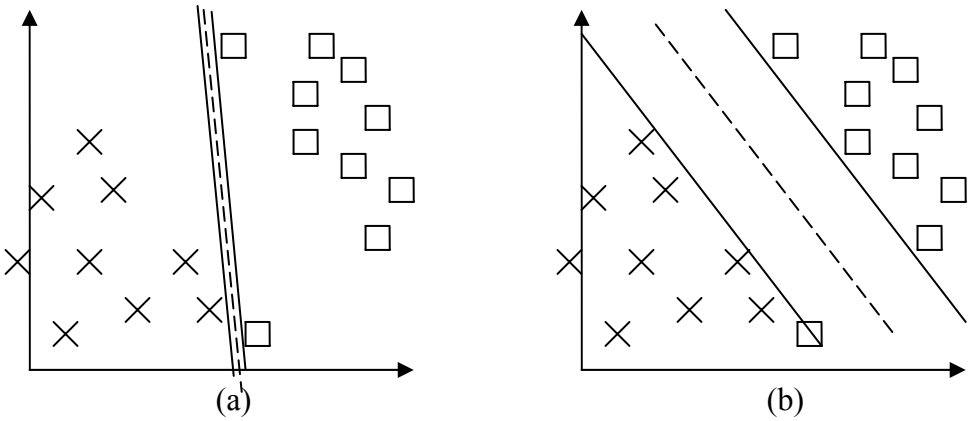


Figure 5.3: (a) Hard margin solution when data contain noise. (b) Soft margin solution when data contain noise.

In order to overcome this problem, the constraints are relaxed by introducing some non-negative variables ζ_i known as the *slack variables* (Cortes and Vapnik, 1995). In other words, some margin errors are allowed, hence achieving a soft margin instead of a hard margin (no margin errors).

$$\langle w \cdot x_i \rangle + b \geq +1 - \zeta_i \quad if \quad y_i = +1 \tag{5.25}$$

$$\langle w \cdot x_i \rangle + b \geq -1 + \zeta_i \quad if \quad y_i = -1 \tag{5.26}$$

$$for \ \zeta_i \geq 0, \quad i = 1, \ldots, n$$

These two equations can be put together as:

$$y_i\left(\langle w \cdot x_i \rangle + b\right) \geq 1 - \zeta_i, \qquad i = 1, \ldots, n \tag{5.27}$$

The generalization performance is improved by maintaining the right balance between the capacity (soft-margin parameter) and error. There exist bounds on generalization errors which minimize the selected (1 or 2) norm of the slack variables (Cristianini and Shawe-Taylor, 2000). The objective function is therefore changed to,

$$\frac{1}{2}\langle w \cdot w \rangle + C\sum_{i=1}^{n}\zeta_i^p \tag{5.28}$$

where p is an integer. For $p = 1$, we get the 1-norm soft margin optimization problem and for $p = 2$, we get the 2-norm soft margin optimization problem. C is a trade-off parameter. A right value of C produces a classier with good generalization. A hyperplane which tolerates training errors is known as a *generalized optimal hyperplane*. The corresponding optimization problem is: Given a set of training instances $\{(x_1, y_1), (x_2, y_2), \ldots, (x_n, y_n)\}$,

$$\textit{Minimize} \qquad \frac{1}{2}\langle w \cdot w \rangle + C\sum_{i=1}^{n}\zeta_i \tag{5.29}$$

Subject to:

$$y_i\left(\langle w \cdot x_i \rangle + b\right) \geq 1 - \zeta_i$$
$$\zeta_i \geq 0, \quad \textit{for all} \quad i = 1, \ldots, n$$

The corresponding dual is given by

$$\textit{Maximize} \qquad \sum_{i=1}^{n}\alpha_i - \frac{1}{2}\sum_{i,j=1}^{n}\alpha_i\alpha_j y_i y_j \langle x_i \cdot x_j \rangle \tag{5.30}$$

Subject to:

$$\sum_{i=1}^{n}\alpha_i y_i = 0$$
$$0 \leq \alpha_i \leq C \quad \textit{for all} \quad i = 1, \ldots, n$$

It is similar to the optimal hyperplane except there is an upper bound on the value of α_i. We can give the general kernel version:

$$Maximize \qquad \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{n} \alpha_i \alpha_j y_i y_j k \left\langle x_i \cdot x_j \right\rangle \tag{5.30}$$

Subject to:

$$\sum_{i=1}^{n} \alpha_i y_i = 0$$
$$0 \le \alpha_i \le C \quad for\ all \quad i = 1, \ldots, n$$

Note that the threshold b is related to the constraint $\sum_{i=1}^{n} \alpha_i y_i = 0$. We will describe a simple on-line SVM algorithm (Friess *et al*., 1998) by fixing the threshold equal to zero in the case the constraint $\sum_{i=1}^{n} \alpha_i y_i = 0$ is not needed.

5.4 Features Representation

The construction of an appropriate feature space that describes the training data is essential for any supervised machine learning system. In the context of protein-protein interactions, it is believed that the likelihood of two proteins to interact with each other is associated with their structural domain composition (Kim *et al*., 2003; Pawson and Nash, 2003; Ng *et al*., 2003). It is also assumed that the hydrophobic effects drive protein-protein interactions (Chung *et al*., 2004; Uetz and Vollert, 2005). For these reasons, this study investigates the applicability of the domain structure and hydrophobicity properties as protein features to facilitate the prediction of protein-protein interactions using the support vector machines.

The domain data was retrieved from the database of protein families (PFAM) database. PFAM is a reliable collection of multiple sequence alignments of protein

families and profile hidden Markov models (Bateman *et al*., 2004). The current version 10.0 contains 6190 fully annotated PFAM-A families. PFAM-B provides additional PRODOM-generated alignments of sequence clusters in SWISSPROT and TrEMBL that are not modeled in PFAM-A.

When the domain information is used, the dimension size of the feature vector becomes the number of domains appeared in all the yeast proteins. The feature vector for each protein was thus formulated as:

$$x\left(p\right)=\left\{d_1,d_2,\ldots,d_i,\ldots,d_n\right\} \tag{5.31}$$

where $d_i = m$ when the protein p has m pieces of domain d_i, and $d_i = 0$ otherwise.

This formula allows the effect of multiple domains to be taken into account. Another representation is by using domain scores that is calculated by PFAM. In this case d_i can be calculated as following:

$$d_i = \sum_{j=1}^{k} S_{i,j} \tag{5.32}$$

where $S_{i,j}$ is the score of the domain i in the location j, and k is the number of the occurrence of domain i in the protein p. In order to scale the feature value to the interval [-1,1], we use the following formula.

$$d_i = \sum_{j=1}^{k} (6 - (\ln(S_{i,j} + 0.1)) \tag{5.33}$$

In the same manner, the amino acid hydrophobicity properties can be used to construct the feature vectors for SVM. The amino acids hydrophobicity properties are obtained from (Hopp and Woods, 1981). The hydrophobicity features can be represented in feature vector as:

$$x\left(p\right)=\left\{h_1,h_2,\ldots,h_i,\ldots,h_r\right\} \tag{5.34}$$

where *r* is the number of amino acid in the protein *p*, $h_i=1$ when the amino acid is hydrophobic and $h_i=1$ when the amino acid is hydrophilic. We also consider the case where the hydrophobicity scale can be included in the feature vector by replacing the amino acid with its correspondent hydrophobicity value.

Using the above described four feature representations, we constructed four training set (domains, domains with scores, hydrophobicity, and hydrophobicity with scale). Each training example is a pair of interacting proteins (positive example) or a pair of proteins known or presumed not to interact (negative example).

5.5 Materials and Implementations

The performance of our technique will be tested on dataset obtained from the database of interacting proteins (DIP) (Xenarios *et al*., 2002). In the following subsections, we will describe in details this dataset used in this research as well as the experiment data preparation processes.

5.5.1 Data Sets

The DIP database was developed to store and organize information on binary protein–protein interactions that was retrieved from individual research articles. The DIP database provides sets of manually curated protein-protein interactions in Saccharomyces cerevisiae. It combines information from a variety of sources to create a single, consistent set of protein-protein interactions. The data stored within the DIP database were curated, both, manually by expert curators and also automatically using computational approaches that utilize the knowledge about the

protein-protein interaction networks extracted from the most reliable, core subset of the DIP data.

At the time of experiments, DIP contains 4749 proteins involved in 15675 interactions for which there is domain information. DIP also provides a high quality core set of 2609 yeast proteins that are involved in 6355 interactions which have been determined by at least one small-scale experiment or at least two independent experiments and predicted as positive by a scoring system (Deane *et al*., 2002).

The data format is shown in Figure 5.4. It describes the interactions between protein pairs. The first column gives the DIP ID of the first protein pair. The letter N at the end of the ID is refereeing to "node" where the proteins in the interactions network are the nodes. It is followed by the protein standard and systematic name. Then the other protein in the pair is described similarly in the followed three columns. The last column represents the DIP ID for this interactions and it ends with the letter E, referring to "edge" in the interactions network.

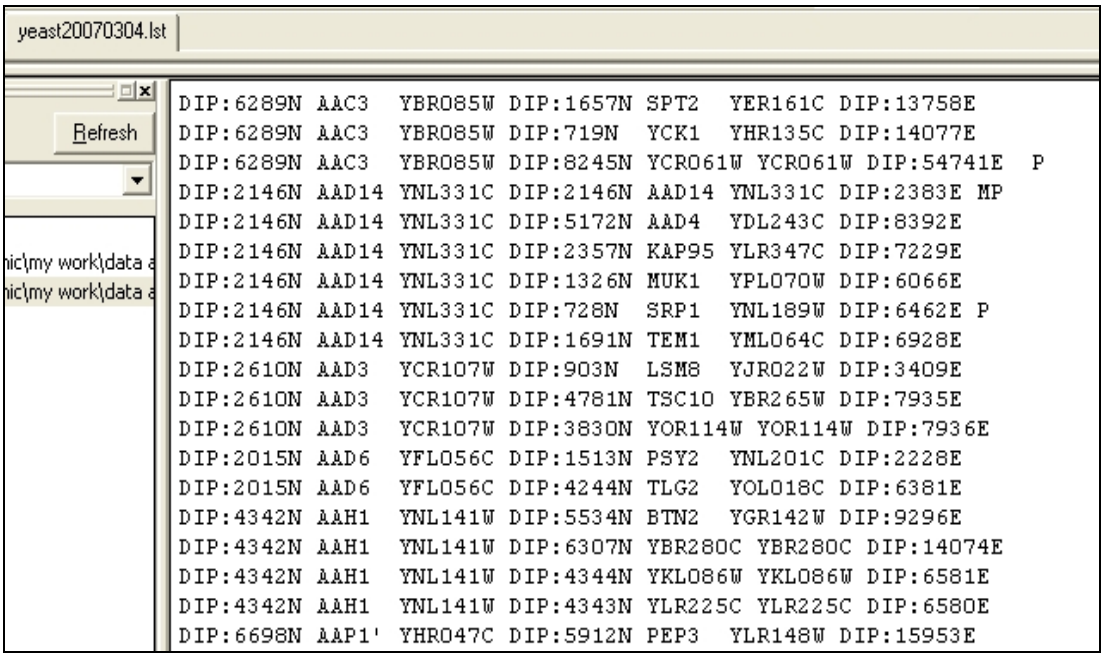


Figure 5.4: Part of the protein-protein interactions list from DIP.

Using a Perl program, this file was transformed to a format of interacting protein as shown in Figure 5.5 The first column is the sequence name for the first protein in the interactions pair and the second column is the sequence name for the second protein in the interactions pair.

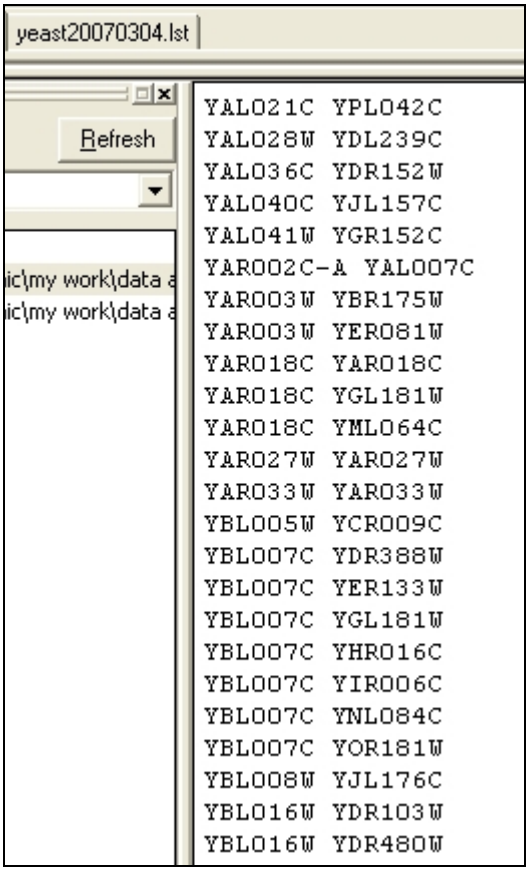


Figure 5.5: Part of the protein-protein interactions list with sequences name only.

The proteins sequence information is needed in this research in order to elucidate the domain structure of the proteins involved in the interaction and to represent the amino acid hydrophobicity in the feature vectors. The proteins sequences files were obtained for the Saccharomyces Genome Database (SGD) (Hong *et al*., 2005). The SGD project collects information and maintains a database of the molecular biology of the yeast Saccharomyces cerevisiae. This database includes a variety of genomic and biological information and is maintained and updated by SGD curators. Figure 5.6 shows part of the protein sequence file that was obtained from SGD. It uses the FASTA format. FASTA format is a text-based

format for representing either nucleic acid sequences or protein sequences, in which base pairs or protein residues are represented using single-letter codes. The format also allows for sequence names and comments to precede the sequences.

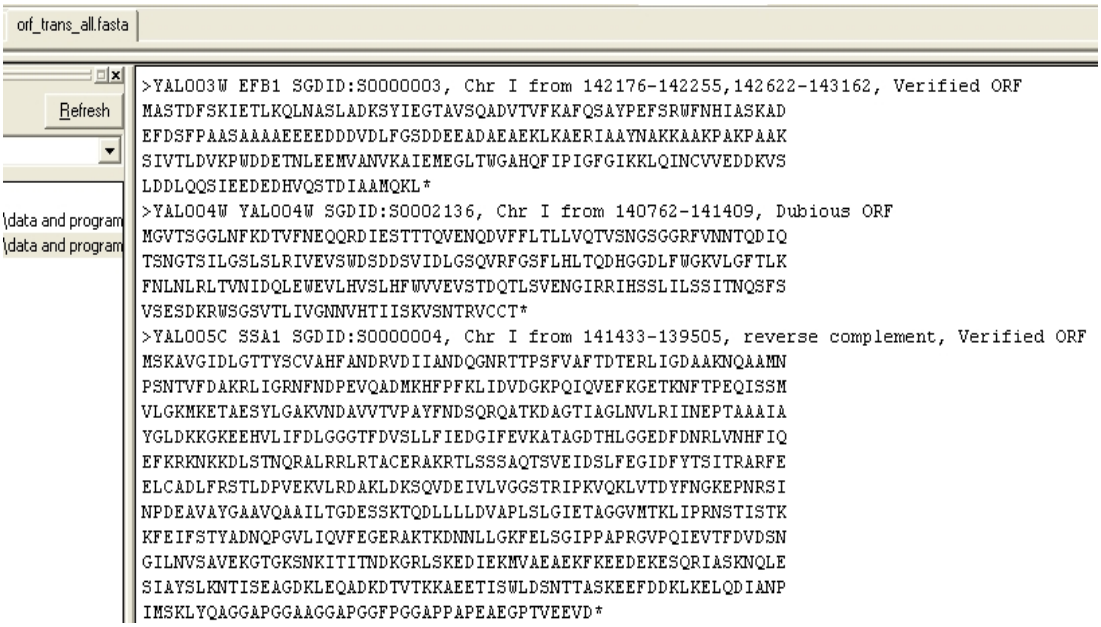


Figure 5.6: Part of the protein sequence file.

5.5.2 Data Preprocessing

Since proteins domains are highly informative for the protein-protein interaction, we used the domain structure of a protein as the main feature of the sequence. We focused on domain data retrieved from the PFAM database which is a reliable collection of multiple sequence alignments of protein families and profile hidden Markov models (Bateman *et al*., 2004). In order to elucidate the PFAM domain structure in the yeast proteins, we first obtain all sequences of yeast proteins from SGD. Given that sequence file, we then run InterProScan (Mulder *et al*., 2003) to examine which PFAM domains appear in each protein. We used the stand-alone version of InterProScan. Part of the result file is shown in Figure 5.7.

```
<protein id="Q0065" length="544" crc64="A77CD9ADBDCA6465" >
<interpro id="IPR000883" name="Cytochrome c oxidase, subunit I"
type="Family">
   <child_list>
      <rel_ref ipr_ref="IPR004677"/>
   </child_list>
   <match id="PF00115" name="COX1" dbname="PFAM">
      <location start="5" end="339" score="8.2e-67" status="T"
evidence="HMMPfam" />
   </match>
</interpro>
<interpro id="IPR001982" name="Homing endonuclease, LAGLIDADG/HNH"
type="Domain">
   <match id="PF00961" name="LAGLIDADG_1" dbname="PFAM">
      <location start="316" end="403" score="6.4e-22" status="T"
evidence="HMMPfam" />
      <location start="422" end="515" score="3.2e-11" status="T"
evidence="HMMPfam" />
   </match>
</interpro>
</protein>
```

Figure 5.7: Part of the protein domains file.

From the output file of InterProScan, we list up all PFAM domains that appear in yeast proteins and index them. The order of this list is not important as long we keep it through the whole procedure. The number of all domains listed and indexed in this way is considered the dimension size of the feature vector, and the index of each PFAM domain within the list now indicates one of the elements in a feature vector. Figure 5.8 shows an example of protein domains that appears in yeast genome. The first column represents a protein whereas the following columns represent the domains that appear in the protein.

The next step is to construct a feature vector for each protein. For example, if a protein has domain A and B which happened to be indexed 12 and 56 respectively in the above step, then we assign "1" to the 12th and 56th elements in the feature vector, and "0" to all the other elements. Next we focus on the protein pair to be used for SVM training and testing. The assembling of feature vector for each protein pair can be done by concatenating the feature vectors of proteins constructed in the previous step. When hydrophobicity is used, each amino acid will be replaced by 1 if

it is hydrophobic and 0 if it is hydrophilic. Two separate training sets for domain and hydrophobicity features have been constructed. Figure 5.9 shows part of the final file where the feature vectors are in SVM format.

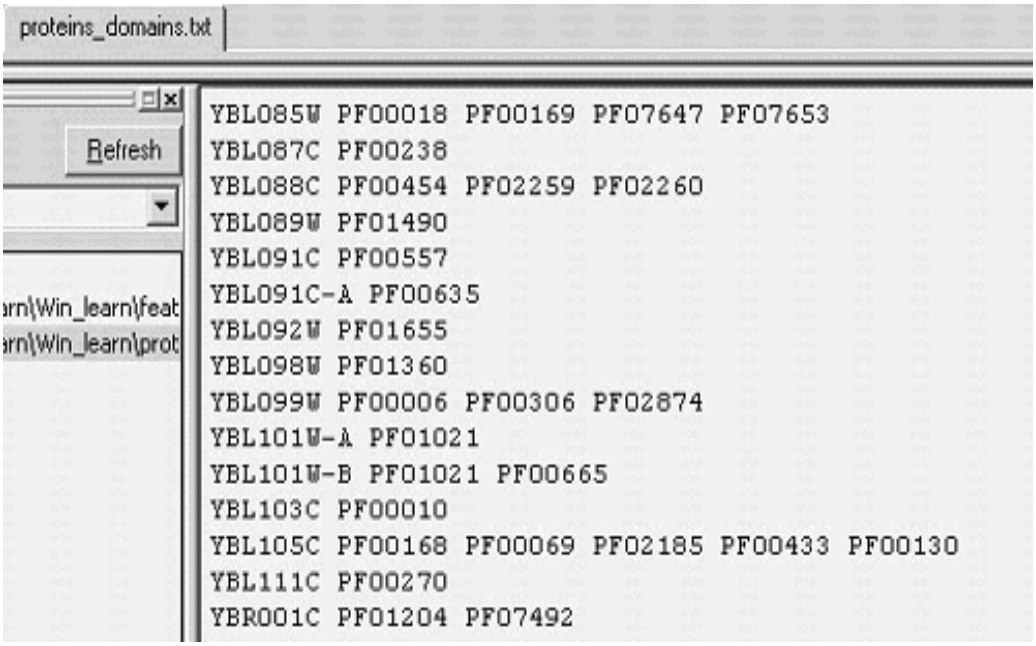


Figure 5.8: Part of the protein domains structure for the yeast genome.

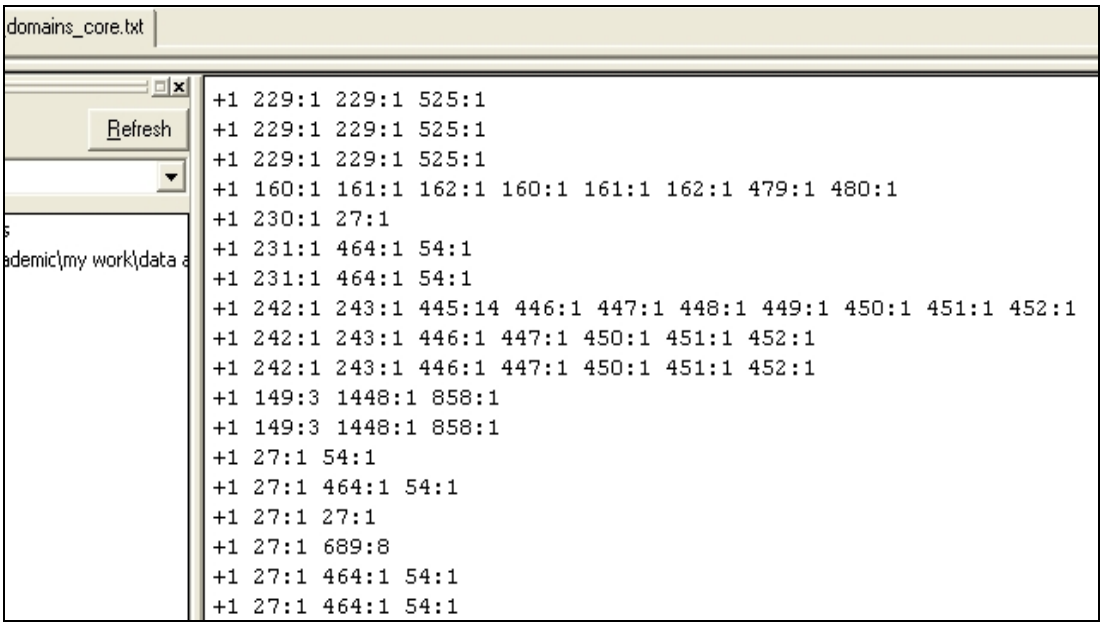


Figure 5.9: Part of the training data file.

5.6 Results and Discussion

We developed programs using Perl for parsing the DIP databases, control of randomization and sampling of records and sequences, and replacing amino acid sequences of interacting proteins with its corresponding feature. To make a positive interaction set, we represent an interaction pair by concatenating feature vectors of each proteins pair that are listed in the DIP-CORE as interacting proteins. For the domain feature we include only the proteins that have structure domains. The resulting positive set for domain feature contains 1879 protein pairs. But when using hydrophobicity feature, all protein in DIP-CORE were included which yielded 3002 protein pairs.

Constructing a negative interaction set is not an easy task. This is due to the fact that there are no experimental data in which protein pairs have confirmed to be non-interacting pairs. As a result, using a random approach to construct the negative data set is an avoidable at this moment. Furthermore, for the purposes of comparing prediction algorithms or feature representation, the resulting inaccuracy will be approximately uniform with respect to each computational method or feature representation. For these reasons, the negative interaction set was constructed by generating random protein pairs. Then, all protein pairs that exist in DIP were eliminated.

This random approach can generate as many as 20202318 potentially negative candidates. Hence, the number of positive protein pairs is quite small compared to that of potentially negative pairs. The excessive potentially negative examples in the training set may lead to yield many false negatives because many of the positive examples are ambiguously discriminative from the negative examples in the feature space. For this reason, a negative interaction set was constructed containing the same number of protein pairs as for the positive interaction set for domain and hydrophobicity features.

In this study, we used the LIBSVM software developed by Chang and Lin, (2001) as a classification tool. The standard radial basis function (RBF) as available in LIBSVM was selected as a kernel function. The RBF kernel is stated as following:

$$K(x_i, x_j) = e^{(\gamma \|x_i - x_j\|^2)} \qquad , \gamma > 0. \tag{5.35}$$

We choose the RBF kernel because it nonlinearly maps samples into a higher dimensional space, so it, unlike the linear kernel, can handle the case when the relation between class labels and attributes is nonlinear (Keerthi and Lin 2003). In addition, the RBF kernel has less numerical difficulties (Chang and Lin, 2001).

Different values of γ in the RBF were systematically tested to optimize the balance between sensitivity and specificity of the prediction. Ten-fold cross-validation was used to obtain the training accuracy. The entire set of training pairs was split into 10 folds so that each fold contained approximately equal number of positive and negative pairs. Each trial involved selecting one fold as a test set, utilizing the remaining nine folds for training our model, and then applying the trained model to the test set. Then the cross-validation accuracy is calculated as in Equation 5.36. Then the average is calculated for the 10 folds.

$$Accuracy = \frac{TP + TN}{TP + FP + TN + FN} \tag{5.36}$$

From Table 5.1 to Table 5.4, the performance of the SVM classifier is presented in respect to a variant threshold. The shadowed row represents the best performance achieved in terms of cross-validation accuracy. The sensitivity and specificity was calculated using Equations (4.1) and (4.2) respectively.

Table 5.1: The classifier performance on domain structure feature using 10-fold cross validation with variant threshold.

Threshold	Sensitivity	Specificity	Cross-Validation Accuracy
0.1	0.13	0.98	0.47
0.2	0.23	0.95	0.57
0.3	0.39	0.91	0.69
0.4	0.51	0.87	0.75
0.5	0.74	0.81	0.79
0.6	0.83	0.73	0.77
0.7	0.89	0.56	0.73
0.8	0.94	0.43	0.70
0.9	0.97	0.23	0.61

Table 5.2: The classifier performance on domain structure with scores feature using 10-fold cross validation with variant threshold.

Threshold	Sensitivity	Specificity	Cross-Validation Accuracy
0.1	0.12	0.93	0.52
0.2	0.25	0.88	0.55
0.3	0.38	0.85	0.61
0.4	0.53	0.83	0.69
0.5	0.68	0.76	0.72
0.6	0.81	0.72	0.76
0.7	0.83	0.61	0.73
0.8	0.87	0.53	0.70
0.9	0.89	0.33	0.61

Table 5.3: The classifier performance on hydrophobicity feature using 10-fold cross validation with variant threshold.

Threshold	Sensitivity	Specificity	Cross-Validation Accuracy
0.1	0.15	0.94	0.52
0.2	0.27	0.89	0.55
0.3	0.41	0.83	0.61
0.4	0.55	0.81	0.69
0.5	0.67	0.77	0.72
0.6	0.82	0.75	0.78
0.7	0.85	0.61	0.73
0.8	0.89	0.55	0.72
0.9	0.92	0.43	0.67

Table 5.4: The classifier performance on hydrophobicity with scale feature using 10-fold cross validation with variant threshold.

Threshold	Sensitivity	Specificity	Cross-Validation Accuracy
0.1	0.27	0.94	0.60
0.2	0.38	0.91	0.64
0.3	0.51	0.87	0.69
0.4	0.63	0.85	0.74
0.5	0.77	0.83	0.79
0.6	0.80	0.75	0.77
0.7	0.85	0.66	0.75
0.8	0.88	0.57	0.72
0.9	0.92	0.38	0.65

The receiver operating characteristic (ROC) is also used to evaluate the results of our experiments. It is a graphical plot of the sensitivity (true positives rate - TPR) vs. 1-specificity (false positives rate- FPR) for a binary classifier system as its discrimination threshold is varied. The area under the ROC curve is called ROC score.

The results of the experiments are summarized in Table 5.5. All experiments reported in this work, run in Redhat Enterprise Linux AS release 3.2 on 1.8 GHz SMP CPUs with 2 GB of memory.

Table 5.5: The overall performance of SVM for predicting PPI using domain and hydrophobicity features.

Feature	Accuracy	ROC score	Running time
Domain	79.4372 %	0.8480	34 seconds
Domain Scores	76.397 %	0.8190	38 seconds
Hydrophobicity	78.6214 %	0.8159	20,571 seconds (5.7 hours)
Hydrophobicity Scales	79.1375 %	0.7716	34,602 seconds (9.6 hours)

When only domain structure was considered as the protein feature without information on domain appearance score, the cross-validation accuracy and ROC score were respectively 79.4372% and 0.8480. When domain scores were included the cross-validation accuracy and ROC score were decreased to 76.397% and 0.8190 respectively. These results indicate that it is not significant to include the domains score information to the feature representation of the protein pairs. It is informative enough to consider only the existence of domains structure in the protein pairs. It is important here to note that the performance of the prediction algorithm is far better than an absolute random approach which has ROC score of 0.5. This indicates that the difference between interacting and non-interacting protein pairs can be learned from the available data.

In the case of hydrophobicity dataset, the cross-validation and ROC score were respectively 78.6214% and 0.8159. We can see from these results that both domain dataset and hydrophobicity dataset have little difference in terms of cross-validation accuracy. On the other hand, ROC score indicates that domain structure is noticeably better than hydrophobic properties (see Figure. 5.10). Another aspect is

the running time for both features. Clearly, when domain structure used, the data set is much smaller than the data set for the hydrophobic properties. Consequently, the running time required for domain structure training data is much less than the running time required for the hydrophobic training data as shown in Table 5.1.

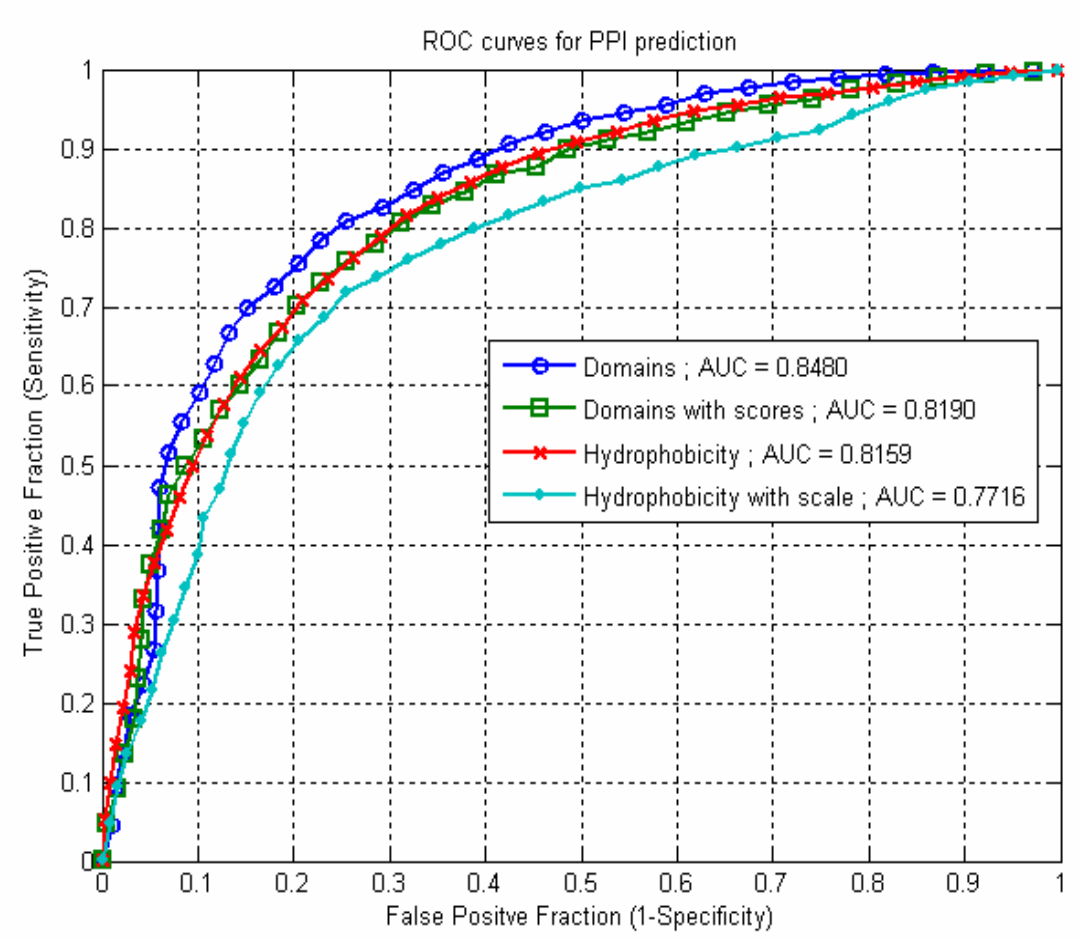


Figure 5.10: The ROC curves and scores for predicting protein-protein interactions.

These results are better and came aligned with the results that have been obtained by Gomez *et al*., (2006) who reported ROC score of 0.818. Whereas our predictor achieved ROC score of 0.848 for domains feature dataset. However, Chung *et al*. (2004) reported accuracy of 94% using hydrophobicity as the protein feature. The reason behind this big difference between our result and their results lies in the approach of constructing the negative interaction dataset. They assign random value to each amino acid in the protein pair sequence. This leads to get new pairs that considered negative interacting pairs and greatly different from the pairs in the

positive interaction set. This leads to simplify the learning task and artificially raise classification accuracy for training data. There is no guarantee, however, that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify. In our work we constructed the negative interactions set by randomly generating non-interacting protein pairs which would be more difficult to distinguish from the positive set than entirely randomizing features values. This makes the learning problem more realistic and ensures that our training accuracy better reflects generalized classification accuracy.

5.7 Summary

The prediction approach explained in this chapter generates a binary decision regarding potential protein-protein interactions based on the domain structure or hydrophobicity properties of the interacting proteins. One difficult challenge in this research as discussed in this chapter is to find negative examples of interacting proteins, i.e., to find non-interacting protein pairs. For negative examples of SVM training and testing, we use a randomizing method. However, finding proper non-interacting protein pairs is important to ensure that prediction system reflects the real world. In conclusion the result in this chapter suggests that protein-protein interactions can be predicted from domain structure with reliable accuracy and acceptable running time. Consequently, these results show the possibility of proceeding directly from the automated identification of a cell's gene products to inference of the protein interaction pairs, facilitating protein function and cellular signaling pathway identification.

CHAPTER 6

ONE-CLASS SUPPORT VECTOR MACHINES FOR PROTEIN-PROTEIN INTERACTIONS PREDICTION

The One-Class Support Vector Machines is proposed in this chapter for the prediction of protein-protein interactions from protein sequence information. Based on the fact that there are no experimentally confirmed non-interacting proteins data, the problem is essentially a one class classification problem. Only one class of data is available and sampled well which is the positive data (interacting proteins). Details on the implementation of the one-class SVM to predict protein-protein interactions are presented in this chapter. At the end of this chapter, the performance of the one-class SVM is presented and discussed.

6.1 Related Work

The completion of the Human Genome Project (HGP) (1990-2003) brought a revolution in biological and bioinformatics research. Currently, researchers have in hand the complete DNA sequences of genomes for many organisms—from microbes to plants to humans. Proteomics research is emerging as the “next step” of genomics.

The proteomics research is extensively concerned with the elucidation of the structure, interactions, and functions of proteins that constitute cells and organisms. Genomics research has already produced a massive quantity of molecular interaction data, contributing to maps of specific cellular networks. In fact, large-scale attempts

have explored the complex network of protein interactions in the *Saccharomyces cerevisiae* (Ito *et al*., 2000; Uetz *et al*., 2000; Newman *et al*., 2000).

In the last few years, the problem of computationally predicting protein-protein interactions has gain a lot of attention. Methods based on the machine learning theory have been proposed (Bock and Gough, 2001; Chung *et al*., 2004; Dohkan *et al*., 2004). Most of these methods address this problem as a binary classification problem. Although, constructing a positive dataset (i.e. pairs of interacting proteins) is relatively an easy task by using one of the available databases of interacting proteins, there is no data on experimentally confirmed non-interacting protein pairs have been made available.

To cope with the unavailability of non-interacting protein pairs, researchers create an artificial negative protein interaction dataset for *Saccharomyces cerevisiae* by randomly generating protein pairs from this organism (Bock and Gough, 2001; Huang *et al*., 2004; Chung *et al*., 2004). The problem with this approach is that in many cases selected “non-interacting” protein pairs will possess features that are substantially different from those typically found in the positive interaction set. This effect may simplify the learning task and artificially raise classification accuracy for training data. There is no guarantee, however, that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify. This effect is clearly observed in Chung *et al*., (2004) work. Using negative dataset that was generated randomly by altering amino acid sequences, they reported 94% prediction accuracy. While Bock and Gough, (2002) and Huang *et al*., (2004) using different random approach to generate negative dataset, reported 80% and 79% respectively. This shows that constructing the negative dataset is a critical problem to be addressed in order to get an accuracy that reflects the reality and does not degrade when presented with unknown data.

Recently, other researchers started to recognize this problem and proposed several solutions to cope with it. Dohkan *et al*., (2004) suggested generating the negative dataset by compiling all possible protein pairs that were not recognized as positive including high-throughput results. All protein pairs that were part of a complex comprising more than two proteins were removed from negative sets, since

those pairs have the possibility interacting physically each other. This filtering yielded 20202318 potentially negative candidates. The number of positive protein pairs is small compared to that of potentially negative pairs. The excessive potentially negative examples in the training set lead to yield many false negatives because many of the positive examples are ambiguously discriminative from the negative examples in the feature space. On the other hand, the insufficient negative examples may yield many false positives and lead to the fluctuation in the prediction performance, since the number of the training samples becomes small. Several data sets positive/negative ration were tried, and finally randomly sampled negative examples that were four times as positive data were used.

Two more requirements to the negative data have been suggested by Huang *et al*. (2004). One of the requirements is that both proteins in each pair should be known to participate in at least one interaction. This requirement was motivated by the assumption that a negative training example where proteins are not known to interact with each other but are both involved in other interactions is harder to separate from positive examples because the proteins in question do possess some sequence or structure features relevant for protein-protein interactions. By contrast, randomly generated protein pairs where one or both proteins are not known to be involved in any interaction have a high chance to lack such features and thus be easier to distinguish from true interactions. The second requirement is that for every positive training example, with high probability, there should be a negative training example possessing the same number of non-zero components in its feature vectors. This is achieved by finding one negative training example (P_i, P_k) for each positive training example (P_i, P_j) where P_k is a randomly chosen interacting protein selected among the proteins with the same number of properties as P_j. If such P_k cannot be found then there will be no negative training example corresponding to (P_i, P_j).

However, all the suggested solution to cope with the unavailability of experimentally non-interacting proteins still needs the artificially random generated negative dataset. In this research, we proposed to deal with problem of predicting protein-protein interactions as a one-class classification problem. This is due to the fact that only data of interacting proteins pairs (positive data) are available and

sampled well. In this respect, we propose a recent method, the one-class support vector machines for the prediction of protein-protein interactions.

6.2 One-Class Classification Problem

The one-class classification problem is a special case from the binary classification problem where only data from one class are available and sampled well. This class is called the target class. The other class which is called the outlier class, can be sampled very sparsely, or can be totally absent. It might be that the outlier class is very hard to measure, or it might be very expensive to do the measurements on these types of objects. For example, in a machine monitoring system where the current condition of a machine is examined, an alarm is raised when the machine shows a problem. Measurements on the normal working conditions of a machine are very cheap and easy to obtain. On the other hand, measurements of outliers would require the destruction of the machine in all possible ways. It is very expensive, if not impossible, to generate all faulty situations (Shin 2005). Only a method trained on just the target data can solve the monitoring problem.

Although the problem of classification is far from solved in practice, the problem of data description or one-class classification is also of interest. The problem in one-class classification is to make a description of a target set of objects and to detect which (new) objects resemble this training set. The boundary between the two classes has to be estimated from data of only the normal, genuine class. The task is to define a boundary around the target class, such that it accepts as much of the target objects as possible, while it minimizes the chance of accepting outlier objects. Figure 6.1 shows an illustration of the target and outlier classes in the one-class classification problem.

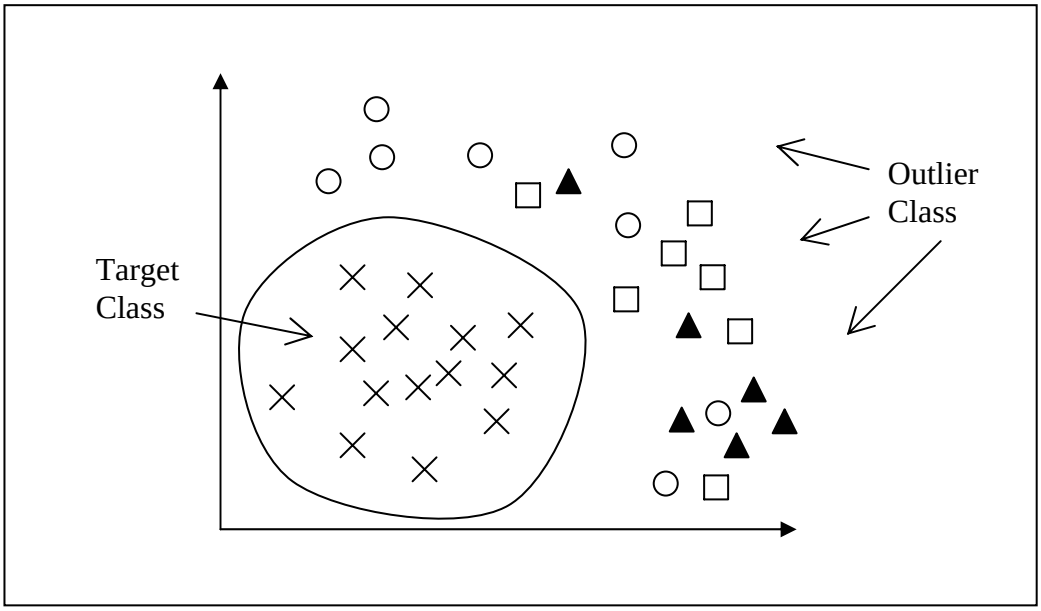


Figure 6.1: Target and outlier classes in the one-class classification problem.

Different terms have been used for the one-class classification problem in the literature. The term one-class classification originates from Moya *et al.*, (1993). However, other terms such as outlier detection (Ritter and Gallegos, 1997), novelty detection (Bishop, 1994) or concept learning (Japkowicz, 1999) were used. The reason behind the use of different terms originates from the different applications to which the one-class classification can be applied.

An obvious application for data description is outlier detection, to detect uncharacteristic objects from a dataset, examples which do not resemble the bulk of the dataset in some way (Ritter and Gallegos, 1997). These outliers in the data can be caused by errors in the measurement of feature values, resulting in an exceptionally large or small feature value in comparison with other training objects. In these cases outlier detection should first be used to detect and reject outliers to avoid unfounded confident classifications.

Another application for data description is for a classification problem where one of the classes is sampled very well, while the other class is severely

undersampled. The measurements on the undersampled class might be very expensive or difficult to obtain. For instance, in a machine monitoring system where the current condition of a machine is examined, an alarm is raised when the machine shows a problem. Measurements on the normal working conditions of a machine are very cheap and easy to obtain. On the other hand, measurements of outliers would require the destruction of the machine in all possible ways. It is very expensive, if not impossible, to generate all faulty situations (Japkowicz, 1995). Only a method trained on just the target data can solve the monitoring problem.

The problem of predicting protein-protein interactions exhibit the characteristics of the one-class classification problem. Several small scale and high-throughput experiments have been developed to detect and identify protein-protein interactions. As a result data on interacting protein are available and sampled well. On the other hand, there are no experiments have been designed to identify proteins that do not interact. This is due to the fact that biologist are not interested in identifying non-interacting proteins because they do not have significant effect on biological processes. Consequently, the data of interacting proteins can be considered the target class and the data of non-interacting proteins can be considered the outlier class.

6.3 One-Class Support Vector Machines

The support vector machines (SVM), which can perform binary classification, has been commonly used as a binary classifier to predict protein-protein interactions. A description of SVM has been presented earlier in Chapter 5. A particular advantage of SVM over other learning algorithms is that it can be analyzed theoretically using concepts from computational learning theory and at the same time can achieve good performance when applied to real problems (Schölkopf and Smola, 2002). SVM has been widely used for several classification problems in the field of computation biology.

The goal of the SVM is to find optimal hyperplane by minimizing an upper bound of the generalization error through maximizing the margin between the separating hyperplane and the data. SVM uses the preprocessing strategy in learning by mapping input space, *X* to a high-dimensional feature space, *F* via a mapping function $\phi(x_i)$. By this mapping, more flexible classifications are obtained. A separating hyperplane is found which maximizes the margin between the separating hyperplane and the nearest training points.

The aim of feature mapping is to find a way of computing the inner product $\langle \phi(x_i) \cdot \phi(x_j) \rangle$ in feature space directly as a function of the original input points (Cristianini and Shawe-Taylor, 2000). The feature space is very high-dimensional space where linear separation becomes much easier than input space.

In general, a pattern classifier uses a hyperplane to separate two classes of patterns based on given examples:

$$S = \{(x_i, y_i)\}_{i=1}^{n} \quad , y_i \in \{-1, +1\} \tag{6.1}$$

The hyperplane is defined by (w, b), where *w* is a weight vector and *b* a bias. The SVMs solution is obtained through maximizing the margin between the separating hyperplane and the data. The linear function of hyperplane can be written as:

$$f(x) = \langle w \cdot x \rangle + b = \sum_{i=1}^{n} w_i x_i + b \tag{6.2}$$

Using a Lagrangian, this optimization problem can be converted into a dual form that is a quadratic programming (QP) problem where the objective function is solely dependent on a set of Lagrange multipliers α_i. The optimization problem is as follows:

$$Minimize \quad \frac{1}{2}\|w\|^2 \tag{6.3}$$

$$Subject\ to: \quad y_i\left(w_i^{\mathrm{T}} x_i + b\right) \geq 1 \qquad for \quad i = 1, 2, \ldots n. \tag{6.4}$$

We can get the maximal margin hyperplane with geometric margin. And then the Lagrangian is as follows:

$$L(\alpha) = \sum_{i=1}^{n} \alpha_i - \frac{1}{2} \sum_{i,j=1}^{n} \alpha_i \alpha_j y_i y_j \left\langle x_i \cdot x_j \right\rangle \tag{6.5}$$

$$Subject\ to: \qquad \alpha_i \geq 0, \quad i = 1, \ldots, n \tag{6.6}$$

$$\sum_{i=1}^{n} \alpha_i y_i = 0 \tag{6.7}$$

The Lagrangian is has to be maximized with respect to α_i. The α_i is Lagrange multiplier and the support vectors lie only close to the hyperplane that have $\alpha_i > 0$. All others points have $\alpha_i = 0$. These support vectors contribute the computing of objective function.

Unlike the standard binary SVM, the one-class SVM treats the origin as the only member of the outlier class (see Figure 6.2). Then using relaxation parameters, it separates the members of the target class from the origin. Then the standard binary SVM techniques are employed.

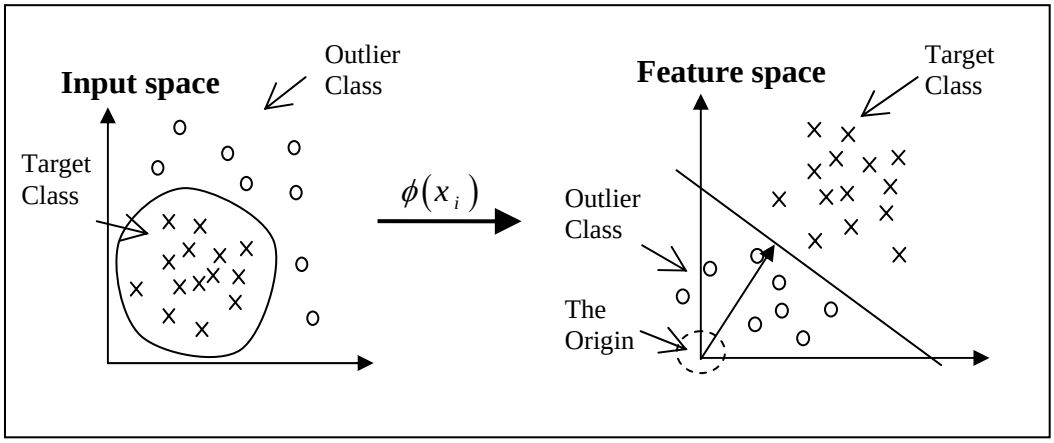


Figure 6.2: Classification in the one-class SVM.

The one-class SVM algorithm works similarly to SVM by mapping input data into a high dimensional feature space (via a kernel) and iteratively finds the maximal margin hyperplane which best separates the training data from the origin.

The feature space points $\phi(x_i),\ldots\phi(x_n)$ are all separable. The distance of the hyperplane is $\langle w \cdot \phi(x_i)\rangle \geq \rho$ and $\rho > 0$. The solution of

$$Minimize_{\ w \in F} \quad \frac{1}{2}\|w\|^2 \tag{6.8}$$

$$Subject\ to: \qquad \langle w \cdot \phi(x_i)\rangle \geq \rho, \qquad i = 1,\ldots,n \tag{6.9}$$

gives the unique hyperplane such that it is closer to the origin than all data and its distance to the origin is maximal among all such hyperplanes. However, not all datasets are linearly separable and it is too difficult to find a canonical hyperplanes quickly. There may be no hyperplane that splits the positive examples from negative examples.

Therefore, error limits ν is to be introduced before the preprocessing of input data. Although this is not canonical hyperplane, it gives acceptable solutions very quickly. In the formulation above, the nonseparable case would correspond to an

infinite solution. By solving for a given constraint $\nu \in (0,1]$ with slack variable ζ_i, most of the data should be separated from the origin by a large margin. Then the new optimization problem can be stated as:

$$\underset{w,\zeta,\rho}{Minimize} \quad \frac{1}{2}\|w\|^2 + \sum_{i=1}^{n}(\zeta_i - \nu\rho) \tag{6.10}$$

$$Subject\ to: \qquad \langle w \cdot \phi(x_i)\rangle \geq \rho - \zeta_i, \qquad i = 1,\ldots,n \tag{6.11}$$

Equation (6.11) can be incorporated into Equation (6.10) by introducing Lagrange multipliers and constructing the Lagrangian with the Lagrange multipliers $\alpha_i \geq 0$ and $\eta_i \geq 0$.

$$L(w,\zeta,\rho) = \frac{1}{2}\|w\|^2 + \sum_{i=1}^{n}(\zeta_i - \nu\rho) + \sum_{i=1}^{n}\alpha_i\left(\rho - \zeta_i - \langle w \cdot \phi(x_i)\rangle\right) - \sum_{i=1}^{n}\eta_i\zeta_i \tag{6.12}$$

For optimality, we have to compute the partial derivatives of L with respect to *w*, ζ, and ρ.

$$\frac{\partial L}{\partial w} = 0 \ \Rightarrow\ w = \sum_{i=1}^{n}\alpha_i\phi(x_i) \tag{6.13}$$

$$\frac{\partial L}{\partial \zeta_i} = 0 \ \Rightarrow\ \alpha_i = 1 - \eta_i \tag{6.14}$$

$$\frac{\partial L}{\partial \rho} = 0 \ \Rightarrow\ n\nu = \sum_{i=1}^{n}\alpha_i \tag{6.15}$$

Then substitute Equations (6.13) and (6.14) into *L* and using kernel function $K(x_i, x_j) = \phi(x_i)\cdot\phi(x_j)$, we obtain the dual problem of Equation (6.10).

$$Minimize \quad \frac{1}{2}\sum_{i,j=1}^{n}\alpha_i\alpha_j K\left(x_i, x_j\right) \tag{6.16}$$

$$Subject\ to: \quad 0 \le \alpha_i \le 1, \quad i = 1,\ldots,n \tag{6.17}$$

$$\sum_{i=1}^{n}\alpha_i = n\nu \tag{6.18}$$

The upper bound in the inequality, Equation (17) limits the influence of objects on the final solution. From the above description the outliers are points x_i which fall on the wrong side of the hyperplane and support vectors are points with $\alpha_i > 0.$ Consequently, ν has two properties. Firstly, at least $n\nu$ points stay on or beyond the margin and at most $n\left(1-\nu\right)$ points stay on the right side of the margin. Secondly, the fraction of outliers is equal to the fraction of support vectors.

6.4 Datasets and Implementation

The construction of an appropriate feature space that describes the training data is essential for any supervised machine learning system. In the context of protein-protein interactions, it is believed that the likelihood of two proteins to interact with each other is associated with their structural domain composition (Kim *et al*., 2002; Pawson *et al*., 2003; Ng *et al*., 2003). It is also believed that hydrophobicity properties of the protein sequence play an important role in mediating protein-protein interactions (Chung *et al*., 2004; Uetz and Vollert, 2005). For these reasons, the implementation of the feature vectors is made using the two features separately. The domain data was retrieved from the PFAM database. The description of the PFAM database is given in Chapter 5 as well as the preparation of domain and hydrophobicity feature vector.

An overview of the implementation framework of the one-class SVM classifier for predicting protein–protein interaction is shown in Figure 6.3. The figure

shows the implementation framework for domain feature, however, similar framework is used for the hydrophobicity feature. Experimentally found protein interactions obtained from Database of Interacting Proteins (DIP) are used for training the one-class SVM classifier. Interaction partners, ‘protein A’ and ‘protein B’, are converted to feature vectors based on domain structure or hydrophobicity properties. Then, predicting if two proteins can interact is done by passing their feature vectors into the one-class SVM classifier which generates the prediction output .

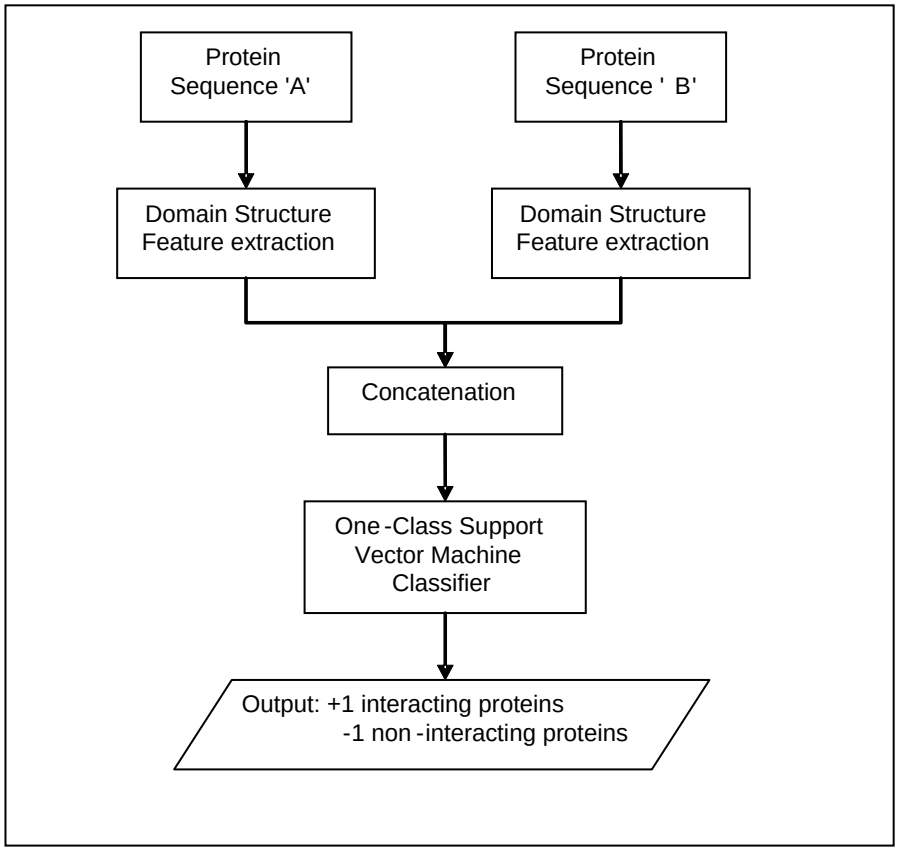


Figure 6.3: The implementation framework for the one-class SVM.

The majority of DIP entries are obtained from combined, non-overlapping data mostly obtained by systematic two-hybrid analyses. More details of DIP datasets is given in Chapter 5. The proteins sequences files were obtained for the Saccharomyces Genome Database (SGD). The SGD project collects information and

maintains a database of the molecular biology of the yeast *Saccharomyces cerevisiae*. The proteins sequence information is needed in this research in order to elucidate the domain structure of the proteins involved in the interaction and to represent the amino acid hydrophobicity in the feature vectors .

The feature vectors files for the domain feature and hydrophobicity feature was developed as described in Section 5.5. In the case of one-class SVM, only positive data was used in the training phase. The classifier should then be used to predict protein-protein interactions from a set of unknown protein pairs. However for testing purpose, we separated a part of the training data to be considered unknown to the classifier. This testing data was also combined with a similar number of random protein pairs that are not included in the DIP.

6.5 Results using Domain Feature

We developed programs using Perl for parsing the DIP databases, sampling of records and sequences, and replacing amino acid sequences of interacting proteins with its corresponding feature. To make a positive interaction set, we represent an interaction pair by concatenating feature vectors of each proteins pair that are listed in the DIP-CORE as interacting proteins. Since we use domain feature we include only the proteins that have structure domains. The resulting positive set for domain feature contains 1879 protein pairs .

In our computational experiment, we employed the LIBSVM (Chang and Lin, 2001) (version 2.5) software and modified it to train and test the one-class SVM proposed in this chapter. This is an integrated software tool for support vector classification, regression, and distribution estimation, which can handle one-class SVM. In order to train the one-class SVM, we examine out the following four standard kernels finding appropriate parameter values:

- Linear Kernel:

$$K(x_i, x_j) = x_i^{\mathrm{T}} x_j \tag{6.19}$$

- Polynomial Kernel

$$K(x_i, x_j) = (\gamma x_i^{\mathrm{T}} x_j + r)^d, \quad \gamma > 0. \tag{6.20}$$

- Radial Basis Function (RBF) Kernel:

$$K(x_i, x_j) = e^{(\gamma \|x_i - x_j\|^2)}, \quad \gamma > 0. \tag{6.21}$$

- Sigmoid :

$$K(x_i, x_j) = tahn(\gamma x_i^{\mathrm{T}} x_j + r) \tag{6.22}$$

where γ (gama), *r*, and *d* are kernel parameters to be set for a specific problem. We carried out our experiments using the above mentioned kernels.

The results of these experiments are given in Figures 6.4 - 6.7. These results indicate that it is informative enough to consider the existence of domains feature in the protein pairs to facilitate the prediction of protein-protein interactions. These results also indicate that the difference between interacting and non-interacting protein pairs can be learned from the available positive data using one-class classifier where no negative data to be randomly generated for the training phase. It is also important to note that the choice of the parameters has a clear impact on the classifier performance. Varying the parameters gives very different predictions accuracy. This suggests that the one-class SVM is very sensitive to the choice of kernel parameters and the error parameter ν (Nu). In addition, the performance on the RBF kernel with γ=64 achieved the best performance and it is steadier than the other kernel

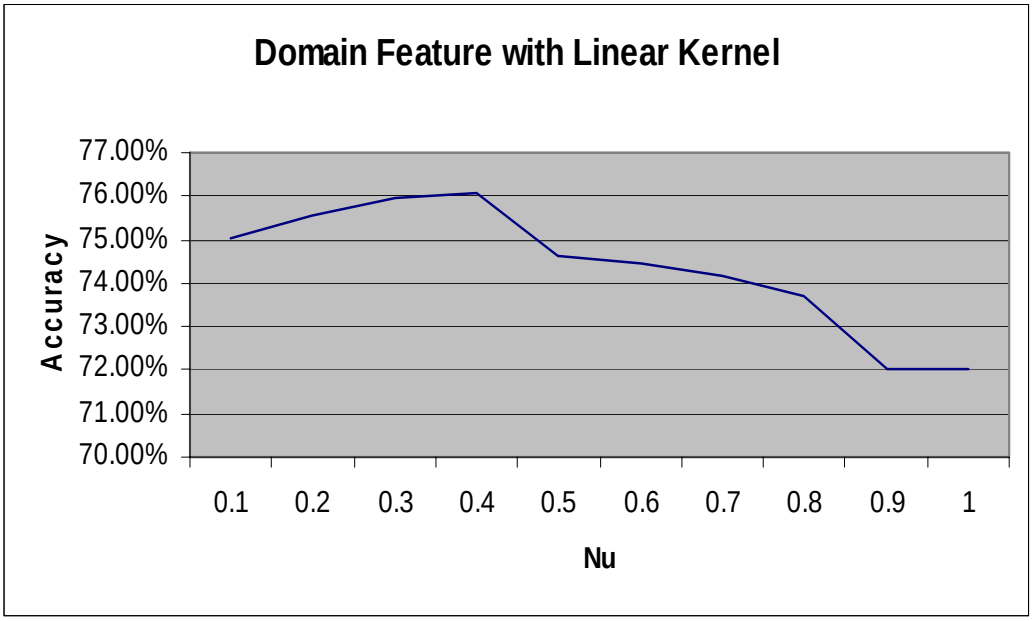


Figure 6.4: The one-class SVM performance using domain feature with the linear kernel.

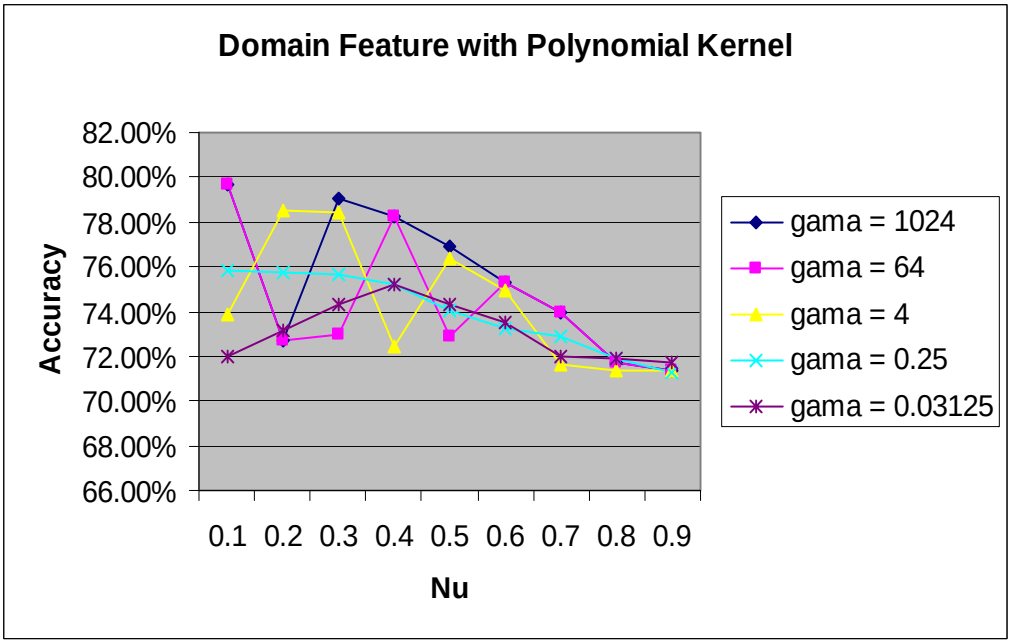


Figure 6.5: The one-class SVM performance using domain feature with the polynomial kernel.

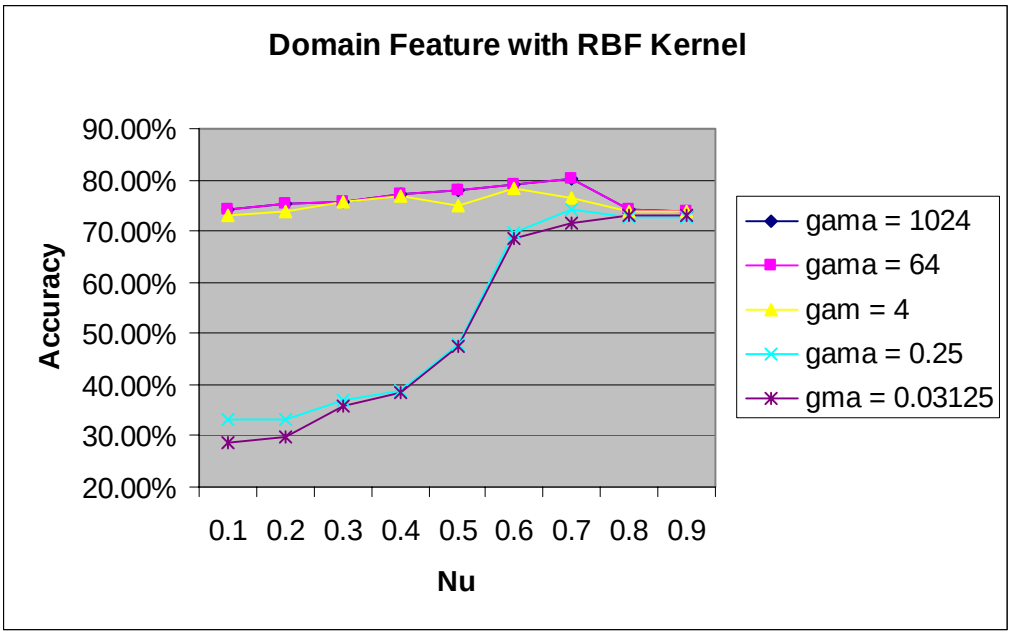


Figure 6.6: The one-class SVM performance using domain feature with the RBF kernel.

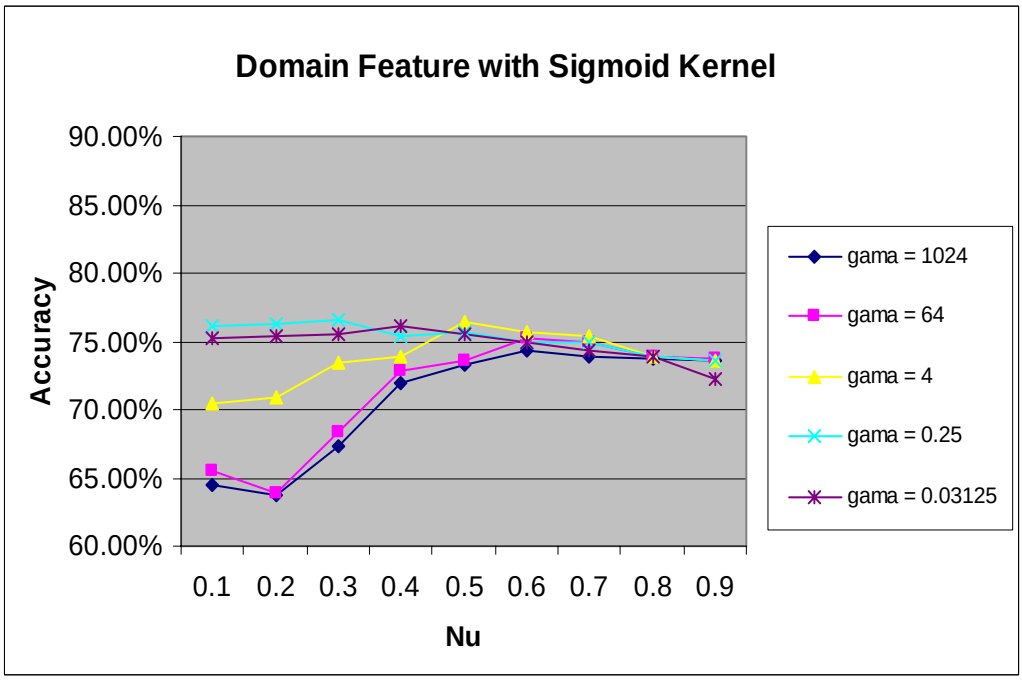


Figure 6.7: The one-class SVM performance using domain feature with the sigmoid kernel.

The results are also summarized in Table 6.1. The results show that the best performance is achieved by the RBF kernel for the domain feature.

Table 6.1: One-Class performance using different kernel with the domain feature.

	Sensitivity	Specificity	Prediction Accuracy
Linear Kernel	0.67	0.83	0.76
Polynomial Kernel	0.71	0.86	0.79
RBF Kernel	0.79	0.80	0.80
Sigmoid Kernel	0.76	0.73	0.74

6.6 Results using Hydrophobicity Feature

We developed programs using Perl for parsing the DIP databases, sampling of records and sequences, and replacing amino acid sequences of interacting proteins with its corresponding hydrophobicity feature. To make a positive interaction set, we represent an interaction pair by concatenating feature vectors of each proteins pair that are listed in the DIP-CORE as interacting proteins. Since we use hydrophobicity feature all protein pairs that is listed in the DIP-CORE is included. The resulting positive set for domain feature contains 3003 protein pairs .

In our computational experiment, we employed the LIBSVM (version 2.5) software as in the previous experiment and modified it to train and test the one-class SVM proposed in this chapter. In order to train the one-class SVM, we examine out the four standard kernels as described in the previous section and the finding appropriate parameter values.

The results of these experiments that use hydrophobicity feature are given in Figures 6.8 - 6.11. These results indicate that protein-protein interactions can be predicted using one-class SVM from hydrophobicity feature with acceptable prediction accuracy. These results also indicate that the difference between interacting and non-interacting protein pairs can be learned from the available positive data using one-class classifier where no negative data to be randomly generated for the training phase. It is also important to note that the choice of the parameters has a clear impact on the classifier performance. Varying the parameters gives very different predictions accuracy. This suggests that the one-class SVM is very sensitive to the choice of kernel parameters and the error parameter ν (Nu).

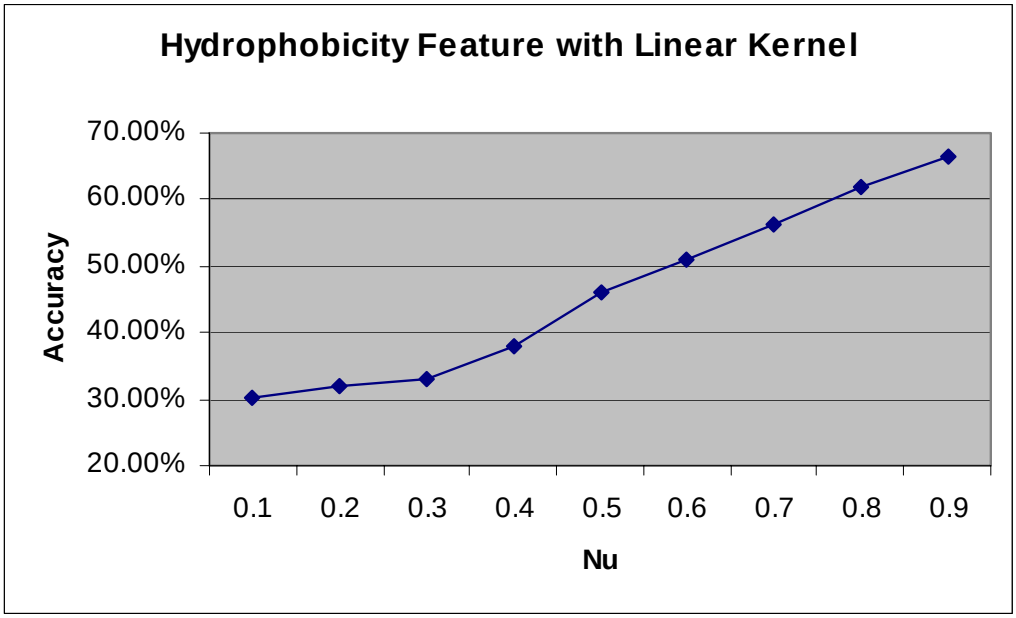


Figure 6.8: The one-class SVM performance using hydrophobicity feature with the linear kernel.

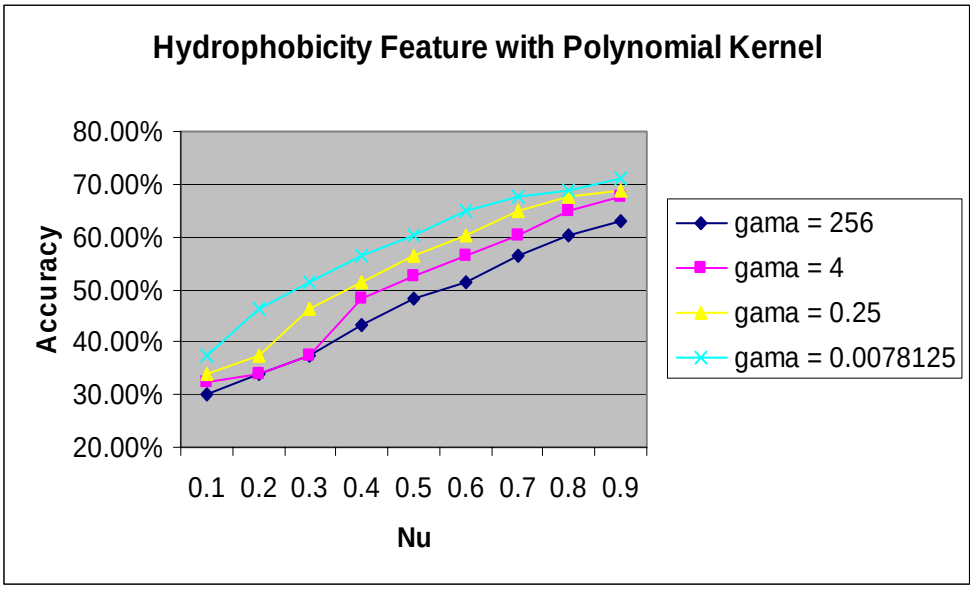


Figure 6.9: The one-class SVM performance using hydrophobicity feature with the polynomial kernel.

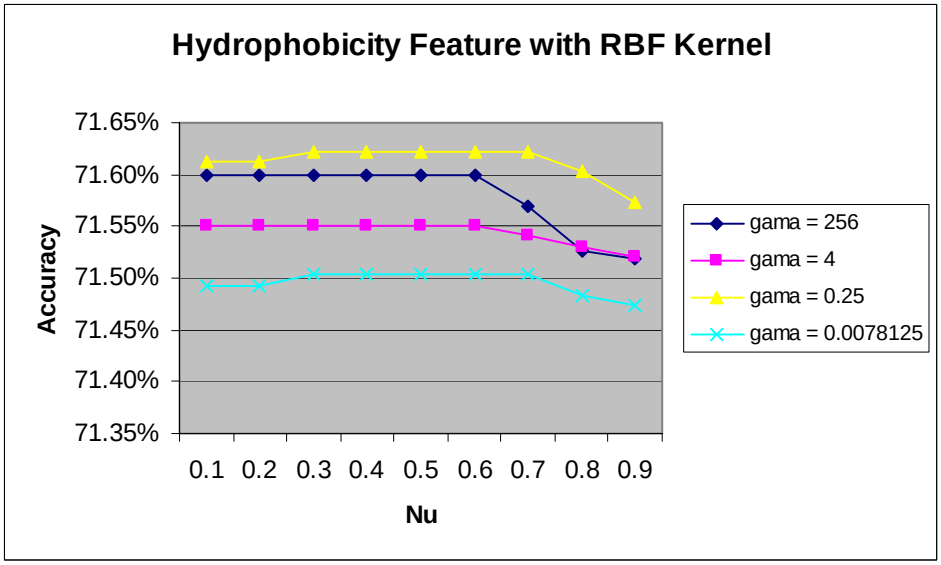


Figure 6.10: The one-class SVM performance using hydrophobicity feature with the RBF kernel.

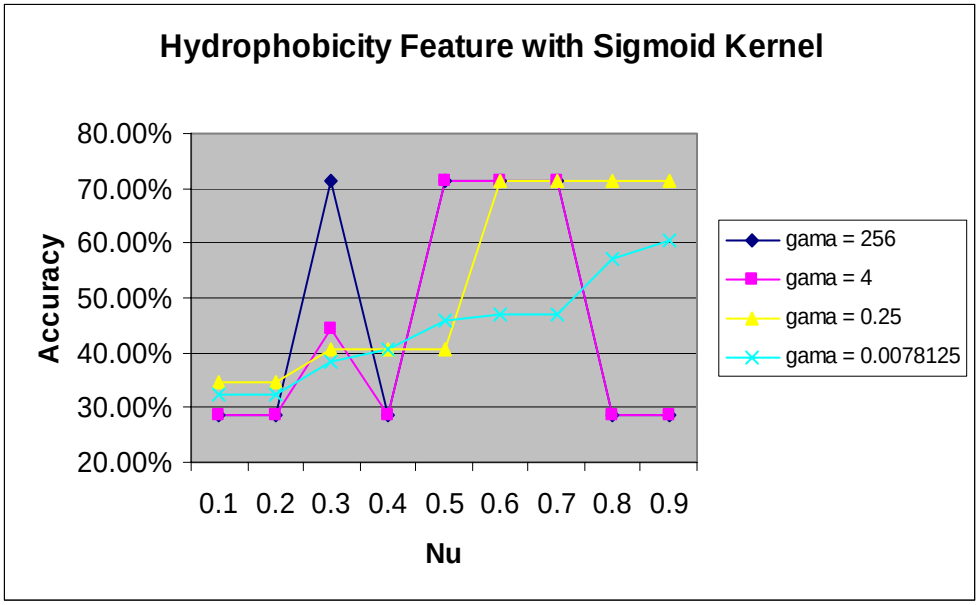


Figure 6.11: The one-class SVM performance using hydrophobicity feature with the sigmoid kernel.

The results of the hydrophobicity feature are also summarized in Table 6.2. The results show that the best prediction accuracy for the hydrophobicity feature was achieved by the polynomial, RBF and sigmoid kernels.

Table 6.2: One-Class performance using different kernel with the domain feature.

	Sensitivity	Specificity	Prediction Accuracy
Linear Kernel	0.55	0.72	0.66
Polynomial Kernel	0.69	0.72	0.71
RBF Kernel	0.70	0.71	0.71
Sigmoid Kernel	0.72	0.69	0.71

6.7 Discussion

Appropriate parameters for one-class SVM with different four kernels are set by the cross-validation process. We can see from this validation process that it is important to choose the appropriate parameters. As shown in Figures 6.4 -6.7, the one-class SVM is very sensitive to the choice of parameters. However, since one-class SVM with linear kernel does not have the parameter γ (gama), we executed the cross-validation process only for parameter ν (Nu). Then the cross-validation accuracy is calculated in each run as the number of corrected prediction divided by the total number of data ((*TP*+*TN*)/(*TP*+*FP*+*TN*+*FP*)). Then the average is calculated for the 10 folds.

The best results were achieved by the RBF kernel (Figure 6.6). Even though, RBF kernel could give as low accuracy as 29% with unsuitable choice of parameters, it achieves around 80% with proper choice of parameters. These results are comparable to the results that have been obtained by Bock and Gough, (2001) and Dohkan *et al*., (2004) with slightly better accuracy.

However, (Chung *et al*., 2004) reported accuracy of 94% using hydrophobicity as the protein feature. The reason behind this big difference between our result and their results lies in the approach of constructing the negative interaction dataset. They assign random value to each amino acid in the protein pair sequence. This leads to get new pairs that considered negative interacting pairs and greatly different from the pairs in the positive interaction set. Consequently, this leads to simplify the learning task and artificially raise classification accuracy for training data. There is no guarantee, however, that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify. In our work we used only positive data in the training set. In this case we don’t need any artificially generated negative data for the training phase. We believe this approach will make the learning problem more realistic and ensure that our training accuracy better reflects generalized classification accuracy. In general, good classification of the training objects is not the main goal, but good classification of new and unseen data is.

6.8 Summary

The problem of predicting protein-protein interactions possesses the features of one-class classification problem where only data from target class (i.e. interacting proteins) are available and sampled well. In this chapter, we have presented the one-class SVM for solving the problem of predicting protein-protein interactions using protein sequence information. Experiments performed on real dataset show that the performance of this method is comparable to standard binary SVM using artificially generated negative set. Of course, the absence of negative information entails a price, and one should not expect as good results as when they are available.

CHAPTER 7

BAYESIAN KERNEL FOR PROTEIN-PROTEIN INTERACTIONS PREDICTION

Kernel functions play an important role for a successful machine learning technique. Choosing the appropriate kernel function can lead to a better accuracy in a binary classifier such as the support vector machines. In this chapter we describe a Bayesian kernel for the support vector machine to predict protein-protein interactions. The use of Bayesian kernel can improve the classifier performance by incorporated the probability characteristic of the available experimental protein-protein interactions data. As shown in this chapter the accuracy of the classifier has been improved using the Bayesian kernel compared to the standard SVM kernels.

7.1 Related Work

Several recent studies have investigated the applicability of Bayesian approaches for the prediction of protein-protein interactions. The Bayesian networks have been successfully applied to predict proteins that are in the same protein complex (Jansen *et al*., 2003). This means that their goal is to predict whether two proteins are in the same complex, not whether they necessarily had direct physical interaction. Having the problem of protein-protein interactions simplified to protein complexes prediction, the construction of gold standard data is feasible by taking the positives from the MIPS catalog of known protein complexes and building the negatives from proteins that are known to be separated in different subcellular compartments. However, to apply Bayesian networks on predicting physical protein-

protein interactions in genome-wide scale, a time complexity and negative examples unavailability will be arisen.

In an attempt to resolve the issues in Bayesian networks approach to predict protein-protein interaction, Yu *et al*., (2005) proposed combining decision trees and Bayesian networks. Their results show that Gene Ontology (GO) annotations can be a useful predictor for protein-protein interactions and that prediction performance can be improved by combining results from both decision trees and Bayesian networks. However, to get a higher quality and more complete interaction map, more types of data have to be combined, including gene expression, phenotype, and protein domains.

In another recent study a method based on the concept of Bayesian inference and implemented via the sum-product algorithm is applied for predicting domain-domain and protein-protein interactions by computing their probabilities conditioned on the measurement results (Sikora *et al*., 2007). The task of calculating these conditional probabilities are formulated as a functional marginalization problem, where the multivariates function to be marginalized naturally factors into simpler local functions. This framework enables the building of probabilistic domain-domain interactions to predict new potential protein-protein interactions based on that information. However, the Bayesian inference approach performance in real data is characterized by low specificity rate. The reason for this limitation of the Bayesian inference with sum-product algorithm, as mentioned by the author, is the higher sensitivity to assumed values of false positive rate (FPR), false negative rate (FNR), and a priori domain-domain interactions probability.

Although Bayesian networks have been applied successfully in a variety of applications, they are an unsuitable representation for complex domains involving many entities that interact with each other (Koller, 1999). Bayesian networks for a given domain involves a pre-specified set of random variables, whose relationship to each other is fixed in advance. Hence, Bayesian networks cannot be used to deal with domains where we might encounter several entities in a variety of configurations.

In order to incorporate the advantages of Bayesian approach in predicting protein-protein interactions and to avoid its time complexity drawback, Bayesian kernel is introduced in the literature. In the following sections, a discussion on Bayesian approaches and kernel methods is presented.

7.2 Bayesian Approach

To understand Bayesian kernel and Bayesian related learning techniques, it is important to understand the Bayesian approach to probability and statistics. In this section, we present a brief introduction to the Bayesian approach to probability and Bayesian learning techniques.

7.2.1 Bayesian Probability

Bayesian probability is an interpretation of probability suggested by Bayesian theory, which holds that the concept of probability can be defined as the degree to which a person believes a proposition. Bayesian theory also suggests that Bayes' theorem can be used as a rule to infer or update the degree of belief in light of new information

In brief, the Bayesian probability of an event A is a person's degree of belief in that event. Whereas a classical probability is a physical property of the world (e.g., the probability that a coin will land heads), a Bayesian probability is a property of the person who assigns the probability (e.g., person's degree of belief that the coin will land heads) (Heckerman, 1998).

The Bayesians probability essentially considers conditional probabilities as more basic than joint probabilities. It is easy to define $P(A|B)$ without reference to the joint probability $P(A,B)$. To see this, the joint and conditional probability formulas can be written as following:

$$P(A,B) = P(A \mid B)P(B) = P(B \mid A)P(A) \tag{7.1}$$

It follows that:

$$P(A \mid B) = \frac{P(B \mid A)P(A)}{P(B)} \tag{7.2}$$

Equation (7.2) represents the Bayes' Rule. It is common to think of Bayes rule in terms of updating our belief about a hypothesis *A* in the light of new evidence *B.* Specifically, our posterior belief *P(A|B)* is calculated by multiplying our prior belief *P(A)* by the likelihood *P(B|A)* that *B* will occur if *A* is true.

7.2.2 Bayesian Networks

Bayesian inference is a statistical inference in which evidence or observations are used to update or to newly infer the probability that a hypothesis may be true. One of the most common techniques to perform Bayesian inference is the Bayesian Networks.

The Bayesian network is a directed acyclic graph which represents independencies embodied in a given joint probability distribution over a set of variables. Nodes can represent any kind of variable such as measured parameters, latent variables or hypothesis. In the Bayesian network graph, nodes correspond to variables of interest and edges between two nodes correspond to a possible dependence between variables.

Over the last decade, the Bayesian network has become a popular representation for encoding uncertain expert knowledge in expert systems (Larrañaga *et al*., 1997). Recently, researchers started to develop methods for learning Bayesian networks from data. The techniques that have been developed are new and still

evolving, but they have been shown to be remarkably effective for some data analysis problems (Niculescu and Mitchell, 2006).

The Bayesian Networks can be represented by a set of variables $X = \{x_1, \cdots, x_2\}$ that encodes a set of conditional independence between these variables. A set *P* of local probability distributions associated with each variable should be defined. The conditional independence and the local probability define the joint probability distribution for *X*. The variable and its corresponding node in the network are denoted by x_i and the parents of node x_i are denoted by pa_i. Given these notations, the joint probability distribution for *X* is given by

$$P(x) = \prod_{i=1}^{n} P(x_i \mid pa_i) \tag{7.3}$$

The probabilities set by a Bayesian networks can be a Bayesian or physical. When prior knowledge is used alone, then the probabilities will be Bayesian. But when learning these networks from data, the probabilities will be physical.

7.3 Kernel Methods

Kernel methods in general and support vector machines in particular have been successfully applied to a number of real-world problems and are now increasingly used to solve various problems in computational biology. They offer different tools to process, analyze, and compare many types of data, and offer state-of-the-art performance in many cases (Vert *et al*., 2004).

During recent years, the machine learning community has shown great interest in Kernel-Based Methods (KM). These methods give state-of-the-art performance by offering an alternative solution by projecting the data into a high dimensional feature space to increase the computational power of the linear learning machines. The support vector machine (SVM) (Vapnik, 1995; Cristianini and

Shawe-Taylor, 2000) is a well known example. However, kernel method is not restricted to SVM. Indeed, it has been pointed out that it can be used to develop nonlinear generalizations of any algorithm that can be cast in terms of dot products, such as principal component analysis (PCA) (Schölkopf *et al*., 1999).

The kernel methods provide a unified framework for machine learning algorithms that enables them to act on different type of data (e.g. strings, vectors, text, etc.) and search for different type of relations (e.g. classifications, regressions, rankings, clusters, etc.). Any kernel method solution comprises two parts: a module that performs the mapping into the embedding or feature space and a learning algorithm designed to discover linear patterns in that space (Shawe-Taylor & Cristianini, 2004).

The building block of these methods is the kernel. The non-dependence of these methods on the dimensionality of the feature space and the flexibility of using any kernel make them a good choice for different classification tasks especially for bioinformatics applications. The learning process of these methods consists of the following stages:

- Map the input data into some higher dimensional space through a nonlinear mapping ϕ. The mapped space is known as the feature space an its denoted by *F* and the mapping is given by:

$$\phi : X \rightarrow F \tag{7.4}$$

- The mapping ϕ may not be known explicitly but can be accessed via the kernel function described later in this chapter.

Figure 7.1, shows the basic idea of kernel methods in which it maps the training data nonlinearly into a higher-dimensional feature space through ϕ, and construct a separating hyperplane with maximum margin there. This yields a nonlinear decision boundary in the input space. The use of a kernel function, allows

the computing of the separating hyperplane without explicitly carrying out the map into the feature space.

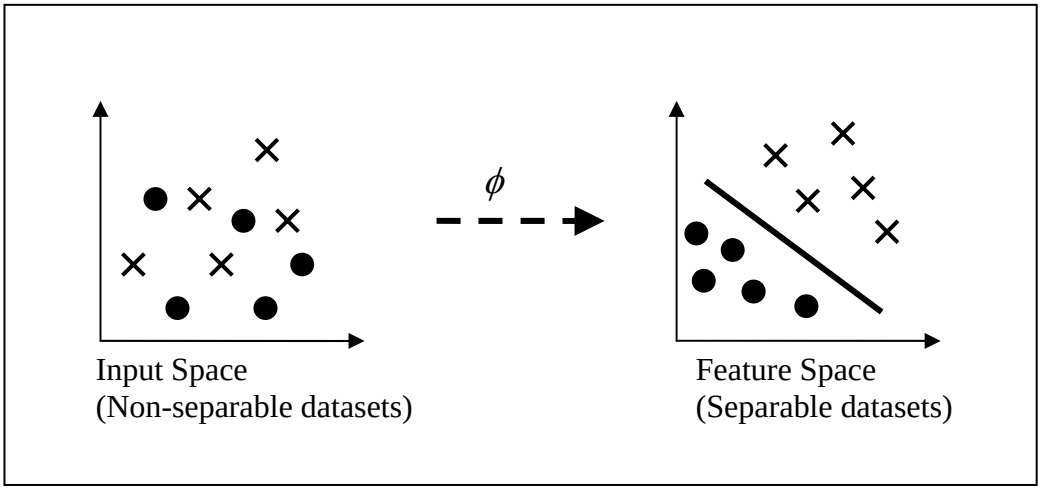


Figure 7.1: Illustration of mapping input data to a feature space.

- Construct a linear classier *f* in the feature space as given by

$$f\ (x) = \langle w \cdot \phi(x) \rangle + b \tag{7.5}$$

Here *w* is the weight vector learned during the training phase and *b* is a bias term. The weight vector is a linear combination of training instances. In other words

$$w = \sum_{i=1}^{n} \alpha_i y_i \phi(x_i) \tag{7.6}$$

where α is a Lagrange multiplier. Substituting the value of *w* yields,

$$f\ (x) = \sum_{i=1}^{n} \alpha_i y_i \langle \phi(x_i) \cdot \phi(x) \rangle + b \tag{7.7}$$

Hence the classifier is constructed only using the inner products between the mapped instances. The kernel trick provides an efficient way to construct such a

classier by providing an efficient method of computing the inner product between mapped instances in the feature space. One does not need to represent the input instances explicitly in the feature space. The kernel function computes the inner product by implicitly mapping the instances to the feature space.

Kernel functions are the basic component shared by all kernel methods. They provide a general framework to represent data. The kernel functions also define how the learning algorithm deals with the data. The kernel function is defined in (Vert *et al.*, 2004) as following:

A function $k : X \times X \to \mathbb{R}$ *is called a positive definite kernel iff it is symmetric, that is,* $k(x, x') = k(x', x)$ *for any two objects* $x, x' \in X$, *and positive definite, that is,*

$$\sum_{i=1}^{n}\sum_{j=1}^{n} c_i c_j k(x_i, x_j) \geq 0 \tag{7.8}$$

for any $n > 0$, *any choice of n objects* $x_1, ..., x_n \in X$, *and any choice of real numbers* $c_1, ..., c_n \in \mathbb{R}$.

In particular, the kernel function have been widely viewed as a function that calculates the inner product between the mapped examples into a feature space is a kernel function that is for any mapping

$$\phi : X \to F,$$
$$k(x_i, x_j) = \langle \phi(x_i) \cdot \phi(x_j) \rangle \tag{7.9}$$

where $x_i, x_j \in X$, and F is any feature space. It can be noted that the kernel computes this inner product by implicitly mapping the examples to the feature space.

The $n \times n$ matrix with entries of the form $k_{i,j} = k(x_i, x_j)$ is known as the Kernel Matrix (KM). Each entry of this matrix represents the inner product between the pairs of the mapped examples. This matrix contains all the information required by the kernel methods. For example, given a kernel *k* and set of *n* vectors the polynomial construction is given by

$$k_{poly}(x_i, x_j) = (\gamma x_i{}^{\mathrm{T}} x_j + r)^d, \qquad \gamma > 0. \tag{7.10}$$

where *d* is the positive integer and γ is a constant. In this case, the feature space corresponding to a degree *d* polynomial kernel includes all products of at most *d* input features. Hence, $k_{poly}(x_i, x_j)$ create images of examples in feature spaces having huge numbers of dimensions.

Furthermore, Gaussian kernels defined feature space with finite number of dimensions and it is given by:

$$k_{gauss}\left(x_i, x_j\right) = \exp\left(\frac{-\left\|x_i - x_j\right\|^2}{2\sigma_2}\right) \tag{7.11}$$

where σ is scaling parameter.

A Gaussian kernel allows an algorithm to learn a linear classifier in an infinite dimensional feature space. Defining a kernel function for an input space is frequently more natural than creating a complicated feature space. Before this route can be followed, however, one must first determine what properties of a function $k(x_i, x_j)$ are necessary to ensure that it is a kernel for some feature space. In fact, any function $k(x_i, x_j)$ that creates a symmetric, positive definite kernel matrix $k_{i,j} = k(x_i, x_j)$ is a valid kernel. In other words, the following Mercer's condition has to be satisfied (Cristianini and Shawe-Taylor, 2000; Schölkopf and Smola, 2002):

$$k(x, y) = \sum_i \phi(x_i)\phi(y_i) \tag{7.12}$$

if and only if, for any $g(x)$ such that

$$\int g(x)^2 dx \qquad \text{is finite} \tag{7.13}$$

then,

$$\int k(x, y) g(x) g(y) dx dy \geq 0 \tag{7.14}$$

New kernels can be designed from given kernel functions. This is because kernel functions are closed under addition and multiplication with a positive constant. The process of designing new kernels by combining simple kernels (Cristianini & Shawe-Taylor, 2000) can be illustrated as following:

Let k_1 and k_2 be kernels over $X \times X$, $X \subseteq \mathbb{R}^n$, $c \in \mathbb{R}^+$. Then the following functions are kernels:

- $k(x, x') = k_1(x, x') + k_2(x, x')$ (7.15)
- $k(x, x') = ck_1(x, x')$ (7.16)
- $k(x, x') = k_1(x, x')k_2(x, x')$ (7.17)

Two points can be drawn from the above discussion. Firstly, the representation of the data as an inner product square matrix does not depend on the nature of the objects to be analyzed. They can be images, molecules, or sequences, and the representation of a data set is always a real-valued square matrix. This suggests that an algorithm developed to process such a matrix can analyze images as well as molecules or sequences, as long as valid kernel functions *k* can be defined.

Secondly, a complete modularity exists between the design of a function *k* to represent data on the one hand, and the design of an algorithm to process the data representations on the other hand. These properties turn out to be of utmost importance in fields like computational biology, where data of different nature need to be integrated and analyzed in a unified framework.

7.4 Bayesian Kernels

The Bayesian kernel exhibits some differences with respect to the standard kernels of SVM. Firstly, in the Bayesian kernel, the prior knowledge can be incorporated into the process of estimation. Secondly, in contrast to the standart kernels of SVM, which simply returns a binary decision, yes or no, a Bayesian kernel returns the probability, $P\left(y=1|x\right)$, that an object *x* belongs to the class of interest indicated by the binary variable *y*. The probability result is more desirable than a simple binary decision as it provides additional information about the certainty of the prediction.

The Relevance Vector Machines (RVM) has been introduced by Tipping (2000) which is a probabilistic sparse kernel method identical in functionality to the SVM. In RVM, a Bayesian approach to learning is adopted. The RVM does not suffer from significant limitations of the SVM. These limitations of the SVM are:

- Predictions are not probabilistic.

- It is necessary to estimate the error or margin trade-off parameter 'C'. This generally entails a cross-validation procedure, which is wasteful both of data and computation

However, the main disadvantage of RVM is in the complexity of the training phase (Tipping, 2000). For large datasets, this makes training considerably slower than for the SVM. Given this fact, designing Bayesian kernel for the SVM would

exhibit the advantages of the Bayesian approach and at the same time avoids the complexity problem of the RVM.

Recently, a Bayesian kernel for the prediction of neuron properties from binary gene profiles has been developed by Fleuret and Gerstner (2005). They provided an analysis of the probabilistic model of the gene amplification process. This analysis yields a similarity measure between two strings of amplified genes that takes the asymmetry of the amplification process into account. This similarity measure was implemented in the form of Bayesian kernel.

This kernel was designed based on the probability of the expressed genes to be the same in both neurons. Given two strings x_i and x_j of amplified gene, the similarity between the strings is quantified as the probability for the expressed genes to be the same in both neurons and it is expressed as following:

$$k\left(x_i, x_j\right) = P\left(Z_i = Z_j \mid X_i = x_i, X_j = x_j\right) \tag{7.18}$$

Here, *X* refers to the random variables on $\{0,1\}^N$ standing for the string of amplified genes (measurement), and *Z* the string of expressed genes (hidden truth). The value 1 stands for “expressed” or “amplified” while 0 stands for “non expressed” or “non amplified”. The only information available here is the value of *X*, and it is required to infer some property of *Z* from the stochastic relation between *X* and *Z*.

The value of Equation (7.18) can be evaluated with the Bayesian rule. It is given that X_i and X_j are independent, and that Z_i and Z_j are independent too. Also, according to amplification model in (Fleuret and Gerstner, 2005), the $X_i^{(l)}$ are conditionally independent. Then:

$$k\left(x_i, x_j\right) = \prod_{l=1}^{N} \kappa_l\left(x_i^{(l)}, x_j^{(l)}\right) \tag{7.19}$$

with,

$$\kappa_l(a,b) = \sum_{c\in\{0,1\}} P\left(Z_i^{(l)} = c \mid X_i^{(l)} = a\right) P\left(Z_j^{(l)} = c \mid X_j^{(l)} = b\right) \tag{7.20}$$

The κ_l can be interpreted as a similarity measure between neurons based on the presence or absence of the *l*-th gene alone. It will take into account the high false negative rate and the absence of false positive. Refer to (Fleuret and Gerstner, 2005) for more details on the Bayesian kernel for the prediction of neuron properties from binary gene profiles.

7.5 Bayesian Kernel for Protein-Protein Interactions Prediction

The development of a Bayesian kernel for protein-protein interactions prediction will facilitate incorporating the prior knowledge via the kernel function. The Bayesian learning is based on the Bayesian rule. In the following, uppercase letters will be used to represent variables and lowercase letters to represent realization. In predicting protein-protein interactions, each observation may be represented by a vector $Z = \{X_1,\ldots,X_m,Y\}$, where $X = \{X_1,\ldots,X_m\}$ is the *m*-dimensional input variable, and *Y* is the output variable taking $\{0,1\}$. Then dataset is represented by:

$$D = \{Z^1,\ldots,Z^n\} = \begin{pmatrix} x_1^1 & x_2^1 & \cdots & x_m^1 & y^1 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ x_1^n & x_2^n & \cdots & x_m^n & y^n \end{pmatrix} \tag{7.21}$$

The conditional probability of Y^i given X^i can be represented as

$$P\left(Y^{i}=1 \mid X_{1}^{i}=x_{1}^{i},\cdots,X_{m}^{i}=x_{m}^{i}\right)=\frac{P\left(Y^{i}=1,X_{1}^{i}=x_{1}^{i},\cdots,X_{m}^{i}=x_{j}^{i}\right)}{P\left(X_{1}^{i}=x_{1}^{i},\cdots,X_{j}^{i}=x_{m}^{i}\right)}$$

$$=\frac{P\left(X_{1}^{i}=x_{1}^{i},\cdots,X_{m}^{i}=x_{m}^{i} \mid Y^{i}=1\right)P\left(Y^{i}=1\right)}{\sum_{y=\{0,1\}}P\left(X_{1}^{i}=x_{1}^{i},\cdots,X_{m}^{i}=x_{m}^{i} \mid Y^{i}=y\right)P\left(Y^{i}=y\right)} \qquad (7.22)$$

and

$$P\left(Y^{i}=0 \mid X_{1}^{i},\cdots,X_{m}^{i}\right)=1-P\left(Y^{i}=1 \mid X_{1}^{i},\cdots,X_{m}^{i}\right) \qquad (7.23)$$

where $P\left(Y^{i}=y^{i}\right)$ is the prior probability of Y^{i} taking value y^{i} and the distribution for the conditional probability $P\left(X_{1}^{i},\cdots,X_{m}^{i} \mid Y^{i}\right)$ can be estimated from the dataset.

Assuming that the input variables are independent for protein-protein interactions dataset, Equation (7.22) can be described as follows:

$$P\left(Y^{i}=1 \mid X_{1}^{i}=x_{1}^{i},\cdots,X_{m}^{i}=x_{m}^{i}\right)$$

$$=\frac{P\left(X_{1}^{i}=x_{1}^{i} \mid Y^{i}=1\right)\cdots P\left(X_{m}^{i}=x_{m}^{i} \mid Y^{i}=1\right)P\left(Y^{i}=1\right)}{\sum_{y=\{0,1\}}P\left(X_{1}^{i}=x_{1}^{i} \mid Y^{i}=y\right)\cdots P\left(X_{m}^{i}=x_{m}^{i} \mid Y^{i}=y\right)P\left(Y^{i}=y\right)} \qquad (7.24)$$

In a similar approach to (Fleuret and Gerstner, 2005) as described in Equations (7.19) and (7.20), we define a Bayesian kernel for protein-protein interactions prediction as:

$$k\left(x^{i},x^{j}\right)=\prod_{l=1}^{m}\kappa_{l}\left(x_{l}^{i},x_{l}^{j}\right) \qquad (7.25)$$

with,

$$\kappa_l\left(x_l^i, x_l^j\right) = \sum_{y\in\{0,1\}} P\left(Y^i = y \mid X_l^i = x_l^i\right) P\left(Y^j = y \mid X_l^j = x_l^j\right) \quad (7.26)$$

The κ_l can be interpreted as a similarity measure between protein pairs based on the *l*-th position of the feature vector. In this experiment we used the domain structure as the protein feature for the representation of the feature vector. For the prior and conditional probability of domains features to facilitate the protein-protein interactions we used the Appearance Probability matrix that was introduced in (Han *et al*., 2004).

The domain combinations and the appearance frequency information of domain combinations are obtained from the interacting and non-interacting sets of protein pairs. The obtained information is stored in the form of a matrix called the Appearance Probability (AP) matrix. When there are n different proteins $\{p_1, p_2, \ldots, p_n\}$ in a given set of protein pairs and the union of domain combinations of proteins contains *m* different domain combinations, $\{d_1, d_2, \ldots, d_m\}$, and then the $m \times m$ AP matrix is constructed. The element AP_{ij} in the matrix represents the appearance probability of domain combination $< d_i, d_j >$ in the given set of protein pairs. Then the conditional probability in Equation (7.26) can be obtained by:

$$P\left(Y^i = 1 \mid X_l^i = x_l^i\right) = \mathrm{AP}_{il} \quad (7.26)$$

7.6 Results and Discussion

In this section, the performance of the SVM classifier with the Bayesian kernel is discussed. The dataset and materials used in this experiment are the same as described earlier in Section 5.5 but only for domain feature.

For constructing the positive interaction set, we represent an interaction pair by concatenating feature vectors of each proteins pair that are listed in the DIP-CORE as interacting proteins. Since we use domain feature we include only the proteins that have structure domains. The resulting positive set for domain feature contains 1879 protein pairs.

As described in Section 5.6, constructing a negative interaction set using a random approach to construct the negative data set is an avoidable at this moment. Furthermore, for the purposes of comparing different kernel methods, the resulting inaccuracy will be approximately uniform with respect to each kernel method. For these reasons, the negative interaction set was constructed by generating random protein pairs. Then, all protein pairs that exist in DIP were eliminated. A negative interaction set was constructed containing the same number of protein pairs.

In our computational experiment, we employed the LIBSVM (version 2.5) software and modified it to use the Bayesian kernel defined in Section 7.5. The performance of the SVM with the Bayesian kernel is compared to the other four standard kernels described in Section 6.5.

Table 7.1 shows the performance of the SVM with Bayesian kernel using domain feature with varied threshold. It shows that there is always a trade off between the sensitivity and specificity. The best cross-validation accuracy is achieved with threshold of 0.5. The specificity is higher than the sensitivity when choosing to have best cross-validation accuracy. This means that the Bayesian kernel can detect the non-interacting protein pairs with a reliable accuracy.

Table 7.1: Bayesian Kernel performance with varied threshold using domain feature.

Threshold	Sensitivity	Specificity	Cross-Validation Accuracy
0.1	0.044	0.991	0.5175
0.2	0.243	0.967	0.605
0.3	0.459	0.941	0.7
0.4	0.621	0.899	0.76
0.5	0.774	0.839	0.8065
0.6	0.844	0.727	0.7855
0.7	0.906	0.596	0.751
0.8	0.954	0.461	0.7075
0.9	0.989	0.253	0.621

The performance of the Bayesian kernel compared to the other four standard kernels is presented in Table 7.2. The Bayesian kernel has significantly improved the prediction accuracy compared to the linear and polynomial kernel. It also has slightly improved the prediction accuracy compared to the RBF and sigmoid kernel. However, it is important to note the Bayesian kernel has the advantage of the probabilistic output over the RBF and sigmoid kernel. It help biologist to conduct further analysis on the predicted interacting proteins pairs with high probability.

Table 7.2: Bayesian Kernel performance compared to the standard kernels using domain feature.

Kernel	Sensitivity	Specificity	Cross-Validation accuracy
Linear Kernel	0.726	0.764	0.768
Polynomial Kernel	0.731	0.787	0.772
RBF Kernel	0.742	0.811	0.793
Sigmoid Kernel	0.751	0.805	0.791
Bayesian Kernel	0.774	0.839	0.8065

The ROC curve is also used to compare the performance of the Bayesian kernel against the standard kernel. Figure 7.2 shows the ROC curve with ROC score for each kernel. The Bayesian kernel perform better than the standard kernels and has higher ROC score.

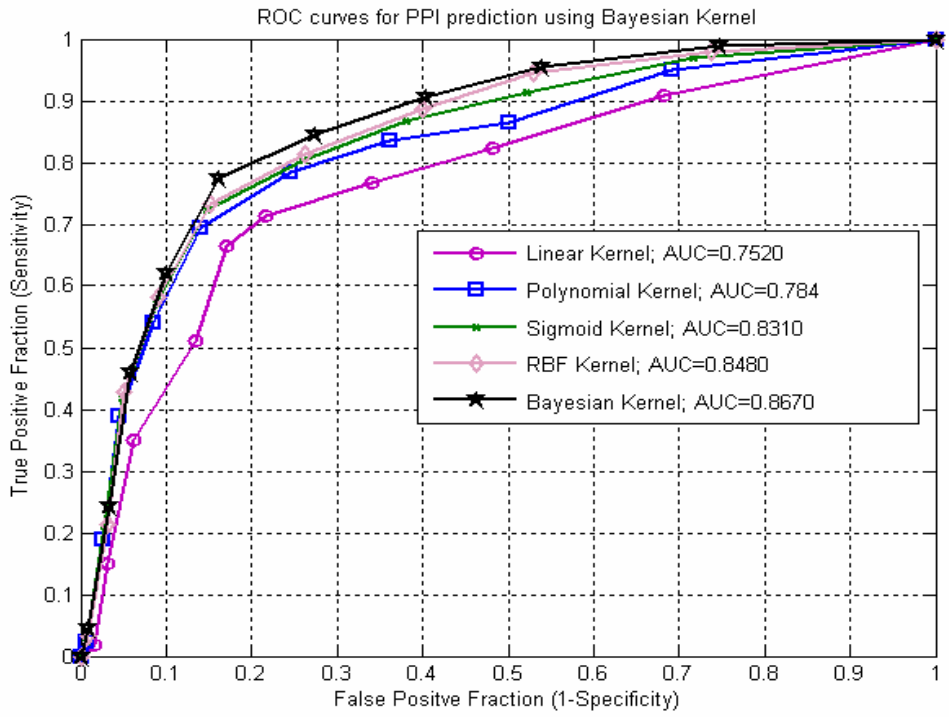


Figure 7.2: The ROC curve for the Bayesian kernel and the standards kernel.

The distribution of the probabilistic output for the Bayesian kernel is shown in Figure 7.3. The Bayesian kernel output a scalar value showing its belief in classification decision. Each protein pair that was predicted either interacting pair or non-interacting pair is assigned a likelihood of the predicted value.

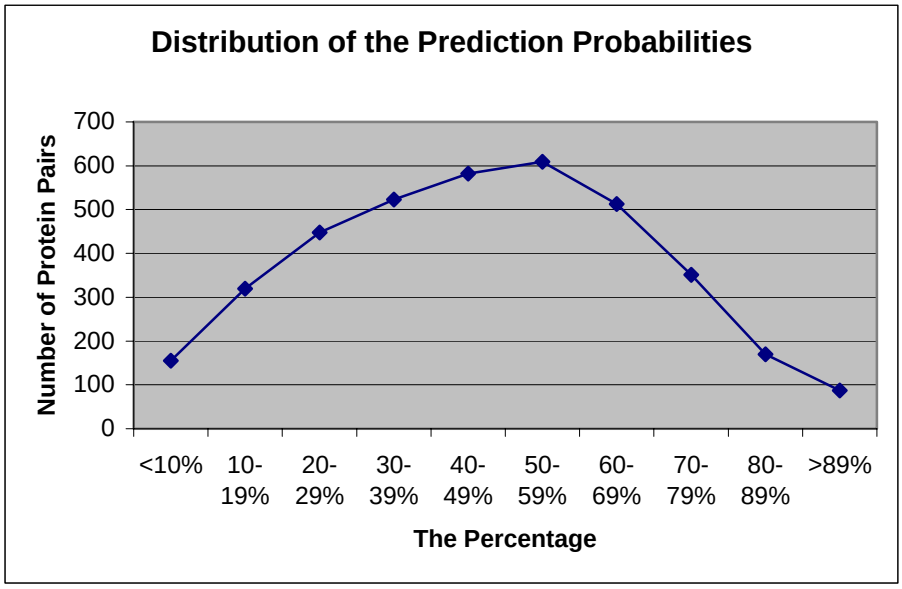


Figure 7.3: The distribution of the probabilistic output for the Bayesian kernel

From Figure 7.3, we can see that the number of protein pairs that have been predicted as interacting pairs with likelihood bigger than 89% is less than 100 pairs which is very small number compared to number interacting protein in the training dataset (1879). However, biologist can carry out experiments to validate the results for the protein pairs that were predicted as interacting pairs with high likelihood. It is time-consuming and costly to carry out experiments to validate the results of all predicted protein pairs.

Comparing protein-protein interaction prediction systems with the other existing systems is always a difficult task. The reason is that, most of the authors used different type of data, experimental setup, and evaluation measures.

Several research studies in the literature reported higher accuracy than it is achieved in this research. The main reason behind these different performances is the construction of the negative dataset for protein-protein interactions. There is no experimentally confirmed non-interacting protein pairs made available by biologist. This contributes to the unavailability of benchmark data that facilitate the comparison of different algorithms. For instance, Chung *et al*. (2004) reported accuracy of 94% by using hydrophobicity as the protein feature. The reason for this high accuracy lies in the approach of constructing the negative interaction dataset. They assign random value to each amino acid in the protein pair sequence. This leads to get new pairs that considered negative interacting pairs and greatly different from the positive interacting pairs in the training dataset. This effect leads to simplify the learning task and artificially raise classification accuracy for training data. There is no guarantee, however, that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify.

In Table 7.3 we compare the performance of the Bayesian kernel developed in this study with some of cited literature that use same datasets and use similar approach in constructing the negative interactions.

Table 7.3: Performance comparison with the cited literature.

Reference	Method	Accuracy	ROC score
Bock & Gough, (2001)	SVM	0.8096	—
Gomez *et al*., (2003)	attraction-repulsion model	—	0.818
Dohkan *et al*., (2004)	SVM	0.788	—
Huang *et al*., (2004)	SVM	0.7957	—
Our Approach	Bayesian Kernel	0.8065	0.8670

As shown in Table 7.3, the Bayesian kernel outperforms most of the related cited literature in terms of ROC score. However, it should be noted that some of the cited literature did not report an ROC score for their method performance. Also in term of prediction accuracy the Bayesian kernel achieve a comparable and slightly better accuracy than most of the cited work.

7.7 Summary

This chapter reports the development and application performance of the Bayesian kernel for the prediction of protein-protein interactions. The Bayesian kernel was developed based on the Bayes' Rule. The performance results of the Bayesian kernel outperformed most of the cited related work with ROC score of 0.8670. However, the comparison with some other works is not feasible due to the fact that different datasets were used. In addition, constructing negative set of non-interacting proteins is still the source of the varied reported accuracy. This is because, until now there is no experimentally confirmed non-interacting proteins dataset. Different cited work use different random method to generate non-interacting protein pairs. In conclusion, the Bayesian kernel provide a better performance as well as probabilistic output that could help biologist to carry out further analysis.

CHAPTER 8

CONCLUSION AND FUTURE WORK

This chapter draws general conclusion of the review of literature, methodology, experimental work, analysis, and the discussion of this research work. The output and results of the developed methods for protein-protein interactions prediction are concluded and summarized in this chapter. This chapter also presents the findings and the contributions of this research. In addition, potential future work is suggested and presented in this chapter.

8.1 Conclusion

It has been established that the rapid development of molecular biology and achievements of modern technology have raised many questions of great bioinformatics interest and there is a growing need to develop, apply and analyze effective and efficient learning methods to improve managing and annotating the novel biological sequences.

Predicting protein-protein interactions is one of the key topics in the post-genomic era. The interactions between proteins are important for many biological functions. Almost all processes in of molecular biology are affected by protein-protein interactions. Therefore, useful methodologies and algorithms for prediction of protein-protein interactions have to be developed and implement. In this thesis several problems of protein-protein interactions prediction have been investigated.

The first objective of this research is to study and investigate different protein sequence feature for the prediction of protein-protein interactions using the support vector machines. In chapter 5 we compare the use of the domain structure and hydrophobicity properties as protein sequence features. This is motivated by several related work in the literature as discussed in chapter 5. In this experiment we consider four features, namely: domain, domain with score, hydrophobicity, and hydrophobicity with scale. The results show that domain feature achieves cross-validation accuracy of 79.4372% and ROC score of 0.8480. This is slightly better accuracy than when using hydrophobicity scale feature. However, domain feature prediction performance is much better in terms ROC score and running time. For domain feature only around 34 seconds is need for the cross-validation experiment while hydrophobicity scale feature takes around 9.6 hours. Other results show that domain score is not important and it is informative enough to consider only the existence of domains structure in the protein pairs. However, hydrophobicity scale feature achieve slightly better accuracy than the hydrophobicity feature but with more running time. It is important here to note that the performance of the prediction system is far better than an absolute random approach which has ROC score of 0.5. This indicates that the difference between interacting and non-interacting protein pairs can be learned from the available data.

The prediction approach reported in Chapter 5 generates a binary decision regarding potential protein-protein interactions based on training set consists of positive dataset (interacting proteins) and negative dataset (non-interacting proteins). Based on the fact that information about protein-protein interactions has been accumulated by various experimental techniques, constructing a dataset of interacting protein is feasible and straight forward. However, there are no experimentally confirmed non-interacting protein pairs. Hence, constructing non-interacting pairs for training the learning system is a challenge. In Chapter 5 experiment, we use a randomizing method to generate negative dataset. This is acceptable for comparing features or algorithms since the error will be uniform on the different features or algorithms.

Given the fact that only information about interacting proteins (positive dataset) are available and sampled well, the problem of predicting protein-protein interaction is essentially one-class classification problem. In Chapter 6, using only positive examples (interacting protein pairs) in training phase, the one-class SVM was implemented and applied. It achieves accuracy of about 80% using the domain features with the RBF kernel. These results indicate that the difference between interacting and non-interacting protein pairs can be learned from the available positive data using one-class classifier. It is also important to note that the choice of the parameters has a clear impact on the classifier performance.

Appropriate parameters for the one-class SVM with the standard four kernels are set by the cross-validation process. The results show that the one-class SVM is very sensitive to the choice of parameters. Even though, the best results were found by the RBF kernel, it could give as low accuracy as 29% with unsuitable choice of parameters.

The results of applying the one-class SVM imply that protein-protein interaction can be predicted using one-class classifier with comparable accuracy to the binary classifiers that use artificially constructed negative dataset. When using randomly generated negative dataset, there is no guarantee that the generalized classification accuracy will not degrade if the predictor is presented with new, previously unseen data which are hard to classify. In our work we used only positive data in the training set. In this case we don’t need any artificially generated negative data for the training phase. We believe this approach makes the learning problem more realistic and ensure that our training accuracy better reflects generalized classification accuracy.

SVM gives its classification output as a binary decision. However, it is desirable to have a probabilistic approach that output a scalar value showing its belief in the classification decision. The Bayesian kernel for SVM gives its output as probabilities. Besides, the Bayesian kernel can improve the classifier performance by incorporated the probability characteristic of the available experimental protein-protein interactions data. Each protein pair that was predicted either interacting pair or non-interacting pair is assigned a likelihood of the predicted value.

The development and implementation of the Bayesian kernel for the SVM was presented in Chapter 7. The Bayesian kernel performs better than the standard kernels and has higher ROC score of 0.8670. The Bayesian kernel outperforms most of the related cited literature in terms of ROC score. However, it should be noted that some of the cited literature did not report an ROC score for their method performance. Also in term of prediction accuracy the Bayesian kernel achieve a comparable and better accuracy compared to most of the cited work.

The overall results indicate that it is informative enough to consider the existence of domains structure in the protein pairs to facilitate the prediction of protein-protein interactions. The Bayesian kernel results with probabilistic output could help biologist to conduct further analysis on the predicted interacting proteins pairs with high likelihood score.

In conclusion the result of this research suggests that protein-protein interactions can be predicted from domain structure and hydrophobicity properties as protein sequence features. Consequently, these results show the possibility of proceeding directly from the automated identification of a cell's gene products to inference of the protein interaction pairs, facilitating protein function and cellular signaling pathway identification.

8.2 Research Contributions

This research focuses on predicting protein-protein interactions with reliable accuracy. The contributions of this research are summarized as follows:

- Investigating and comparing the two main protein sequence features for the prediction of protein-protein interactions using the support vector machines.

- Modeling and solving the problem protein-protein interactions a one-class classification problem using the one-class support vector machines.

- Developing and implementing the Bayesian kernel for SVM to incorporate the probabilistic information about the protein-protein interaction and provide a probabilistic output in the classification decision.

8.3 Future Work

Hopefully, the output of the research can be a motivation for further investigation in the field of protein-protein interactions predictions. In this thesis, protein-protein interactions prediction task successfully performed. In this research some new directions and some important and useful contributions to the efforts to predict protein-protein interactions have been presented. In this section we will outline some of the possible future work directions.

Based on the important issue of feature selection in machine learning, automated methods have to be developed. Suitable features would not only be computationally efficient for the techniques presented in this thesis but will also be a useful contribution in general classification problems. For the sequence features presented and compared in Chapter 5, further investigation of the other possible sequence features is significantly important.

The issue of constructing negative dataset (non-interacting proteins) is a difficult task. This is due to the fact that there are no experimentally confirmed non-interacting proteins have been made available. Hence, investigating different approach to construct the negative set for binary classifiers represent a big challenge. Several methods have been recently investigated in the literature. However, a reliable assessment of the classifiers performance using different approaches for negative dataset construction is needed.

It is also important to note that the available protein-protein interactions data are collected using different experimental techniques. The overlaps between these data are very small. Therefore there is a need for a development of computation techniques that validate the experimental results and assess the reliability of the experimental techniques.

Similar methods of prediction and classification in fields rather than Bioinformatics can successfully utilize variety of techniques and tools used in this research such as the one-class SVM and the Bayesian kernel approach.

Since the research in Bioinformatics field in general and the protein secondary structure prediction domain in particular is increasing rapidly, the need for a “utility and statistical package for Bioinformatics” that successfully arranges data for input and helps in the analysis and assessment of the output becomes crucial. This will save considerable time for the research in Bioinformatics.

8.4 Closing

This chapter concludes and summarizes the research work discussed in this thesis. The chapter also presents and highlights the contributions and findings of this research. Recommendations for further work and future research directions in the domain of this work are also coined and proposed in this chapter. Hopefully this research gives an idea and spreading knowledge in the research field, especially in bioinformatics.

RELATED PUBLICATIONS

Hany Alashwal, Safaai Deris and M. Razib Othman. (2006). One-Class Support Vector Machines for Protein-Protein Interactions Prediction. *The International Journal of Biomedical Sciences*, **1(2)**:120-127.

Nazar Zaki, Safaai Deris and Hany Alashwal. (2006). Protein-Protein Interaction Detection Based on Substring Sensitivity Measure. *The International Journal of Biomedical Sciences*, **1(2)**:148-154.

Hany Alashwal, Safaai Deris and M. Razib Othman. (2006). Comparison of Domain and Hydrophobicity Features for the Prediction of Protein-Protein Interactions using Support Vector Machines. *The International Journal of Information Technology*, **3(1)**:18-24.

Hany Alashwal, Safaai Deris, M. Razib Othman and Mohd Saberi Mohamad. (2006). One-Class Classifier to Predict Protein-Protein Interactions based on Hydrophobicity Properties. *In the International Symposium on Biomedical Engineering* (ISBME'06), November 8-10. Bangkok, Thailand.

Hany Alashwal, Safaai Deris and M. Razib Othman. (2006). Predicting Proteins Interactions from Protein Sequence Features using Support Vector Machines. *In the International Conference on Bioinformatics & Computational Biology* (BioComp'06), June 26-29. Las Vegas, Nevada, USA.

Hany Alashwal, Safaai Deris and M. Razib Othman. (2006). Support Vector Machines for Predicting Protein-Protein Interactions using Domains and Hydrophobicity Features. *In the International Conference on Computing and Informatics* (ICOCI 2006), June 6-8. Kuala Lumpur, Malaysia.

Hany Alashwal, Safaai Deris and M. Razib Othman. (2006). Predicting Protein-Protein Interactions as a One-Class Classification Problem. I*n FSKSM Postgraduate Annual Research Seminar*, May 24-25. Skudai, Johor, Malaysia.

Safaai Deris, Hany Alashwal, Mohd Saberi , Yeo Lee Chin, Muhammad Razib Othman, Yuslina Zakaria, Suhaila Zainudin, Nazar Zaki, Saad Othman, and Satya Arjunan. (2005). Bioinformatics: A Tool for Bio-Knowledge Generation. *In the International Conference on Information Technology and Multimedia* (ICIMU 2005), November 22-24. Kuala Lumpur, Malaysia.

Hany Alashwal and Safaai Deris. (2005). Computational Prediction of Protein-Protein Interactions Based on Protein Domain Structure and Hydrophobic Properties. *In the International Symposium on Bio-Inspired Computing* (BIC05). September 5-7. Johor, Malaysia

Hany Alashwal, Safaai Deris, M. Razib Othman, Azmee Awang and Zalmiyah Zakaria. (2004). Predicting Protein-Protein Interactions from Primary Structure using Support Vector Machines. *In the International Conference on Bioinofromatics* (InCoB2004), September 5-8. Aotea Centre, Auckland, New Zealand.

REFERENCES

Abascal, F. and Valencia, A. (2003). Automatic annotation of protein function based on family identification. *Proteins.* **50**:683-692.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). *Molecular Biology of the Cell* (4th edition). Garland Science.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research.* **25**: 3389-3402.

Attwood, T. K. and Miller, C. J. (2001). Which craft is best in bioinformatics? *Computers and Chemistry.* **25**:327{337.

Bairoch, A. (2000). The ENZYME database in 2000. *Nucleic Acids Res* **28**:304-305.

Bartel, P.L. and Fields, S. (eds) (1997). The yeast two-hybrid system. *In Advances in Molecular Biology*. Oxford University Press, New York.

Bateman A., Coin, L., Durbin, R., Finn, R.D., Hollich, V., Griffiths-Jones, Khanna, S., Marshall, A., Moxon, S.E., Sonnhammer, L.L., Studholme, D.J., Yeats, C. and Eddy S.R. (2004). The Pfam: Protein Families Database. *Nucleic Acids Research Database Issue*. **32**:D138-D141

Ben-Hur, A. and Noble, W.S. (2005). Kernel methods for predicting protein-protein interactions. *Bioinformatics*. **21**:i38–i46.

Bishop, C. (1994). Novelty detection and neural network validation**.** *IEE Proceedings on Vision, Image and Signal Processing*. Special Issue on Applications of Neural Networks. **141(4)**:217–222**.**

Bock, J.R. and Gough, D.A. (2001). Predicting protein-protein interactions from primary structure. *Bioinformatics.* **17(5)**:455-60.

Bork, P., Jensen, L.J., von Mering, C., Ramani, A.K., Lee, I. and Marcotte, E.M. (2004). Protein interaction networks from yeast to human. *Current Opinion Structural Biology*. **14**:292–299.

Boser, B.E., Guyon, I.M., and Vapnik, V.N. (1992). A training algorithm for optimal margin classifiers. *In Haussler, D.(editor) Proceedings of the 5th Annual ACM Workshop on Computational Learning Theory*. Pittsburgh, PA, ACM:144-152.

Boulton, S.J., Gartner, A., Reboul, J., Vaglio, P., Dyson, N., Hill, D.E. and Vidal, M. (2002). Combined functional genomic maps of the C elegans DNA damage response. *Science*. **295**:127-131.

Brown, M. P., Grundy, W. N., Lin, D., Cristianini, N., Sugnet, C. W., Furey, T. S., Ares, M. JR.and Haussler, D. (2000). Knowledge-based analysis of microarray gene expression data by using support vector machines. *Proceedings of National Academy of Sciences USE*. **97**:262-267.

Chang, C.C. and Lin, C.J. (2001). LIBSVM: a library for support vector machines. Software available at http://www.csie.ntu.edu.tw/~cjlin/libsvm. (24/3/2005).

Chung, Y., Kim, G., Hwang, Y. and Park, H. (2004). Predicting Protein-Protein Interactions from One Feature Using SVM. *In IEA/AIE'04 Conf. Proc.* May 17-20. Ottawa, Canada.

Clare, A. and King, R.D. (2002). Machine learning of functional class from phenotype data. *Bioinformatics.* **18:** 160-166.

Cortes, C. and Vapnik, V. (1995). Support vector networks. *Machine Learning*. **20**:273-297.

Costanzo, M.C., Crawford, M.E., Hirschman, J.E., Kranz, J.E., Olsen, P., Robertson, L.S., Skrzypek, M.S., Braun, B.R., Hopkins, K.L., Kondu, P., Lengieza, C., Lew-Smith, J.E., Tillberg, M. and Garrels, J.I. (2001). YPD™, PombePD™, and WormPD™: model organism volumes of the BioKnowledge library, an integrated resource for protein information. *Nucleic Acids Research*. **29:** 75 79.

Craig, R.A. and Liao, L. (2007). Phylogenetic tree information aids supervised learning for predicting protein-protein interaction based on distance matrices. *BMC Bioinformatics*. **8**:6**.**

Cramer, P., Bushnell, D.A., and Kornberg, R.D. (2001). Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science*. **292**:1863–1876.

Deane, C.M., Salwinski, L., Xenarios, I. and Eisenberg, D. (2001). Protein interactions: Two methods for assessment of the reliability of high throughput observations. *Molecular & Cellular Proteomics*. **15**: 349-356.

Deng, M., Zhang, K., Mehta, S., Chen, T. and Sun, F.Z. (2002). Prediction of protein function using protein-protein interaction data. *The first IEEE bioinformatics conference, Stanford University.* CSB2002:197-206.

Dohkan, S., Koike, A. and Takagi, T. (2003). Support Vector Machines for Predicting Protein-Protein Interactions. *Genome Informatics*. **14**:502–503

Dohkan, S., Koike, A. and Takagi, T. (2004). Prediction of protein-protein interactions using Support Vector Machines. *In Proceedings of the Fourth IEEE Symposium on BioInformatics and BioEngineering* (BIBE2004). Taitung, Taiwan. 576-584.

Dohkan, S., Koike, A. and Takagi, T. (2006). Improving the Performance of an SVM-Based Method for Predicting Protein-Protein Interactions. *In Silico Biology*. **6(6)**:515 – 529.

Donaldson, I., Martin, J., de Bruijn, B., Wolting, C., Lay, V., Tuekam, B., Zhang, S., Baskin, B., Bader, G.D., Michalickova, K., Pawson, T., and Hogue, C.W. (2003). PreBIND and Textomy--mining the biomedical literature for protein-protein interactions using a support vector machine. *BMC Bioinformatics*. **4**:11.

Eisen, M., Spellman, P., Brown, P. and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *PNAS*. **95**:14863-14868.

Eisenberg, D., Marcotte, E. M., Xenarios, I. and Yeates, T.O. (2000). Protein function in the post-genomic era. *Nature*. **405**: 823-826.

Eisenberg, D., Schwarz, E., Komaromy, M. and Wall, R. (1984). Analysis of membrane and surface protein sequences with the hydrophobic moment plot. *J. Mol. Biol.* **179**:125-142.

Enright, A.J., Iliopoulos, I., Kyrpides, N.C., and Ouzounis, C.A. (1999). Protein interaction maps for complete genomes based on gene fusion events. *Nature*. **402**:86–90.

Fleuret, F. and Gerstner, W. (2005). A Bayesian Kernel for the Prediction of Neuron Properties from Binary Gene Profiles. *Proceedings of the IEEE International Conference on Machine Learning and Applications*. Special session Applications of Machine Learning in Medicine and Biology (ICMLA):129–134.

Friess, T., Cristianini, N. and Campbell, C. (1998). The kernel-adatron a fast and simple training procedure for support vector machines. *In Shavlik, J.(editor) Proceedings of the 15th International Conference on Machine Learning*. July 24-27. Madison, Wisconsin USA.

Gallet, X., Charloteaux, B., Thomas, A. and Brasseur, R. (2000). A fast method to predict protein interaction sites from sequences. *J. Mol. Biol*. **302**:917-926.

Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M. et al . (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature.* **415**:141-147

Gharakhanian, E., Takahashi, J., Clever, J., and Kasamatsu, H. (1998). In vitro Assay for Protein-Protein Interaction: Carboxyl-Terminal 40 Residues of Simian Virus 40 Structural Protein VP3 Contain a Determinant for Interaction with VP1. *PNAS* **85(18)**:6607-6611.

Goffard, N., Garcia,V., Iragne, F., Groppi, A. and De Daruvar,A. (2003). IPPRED: Server for proteins interactions inference. *Bioinformatics*. **19**:903–904

Gomez, S. M., Noble, W.S. and Rzhetsky, A. (2003). Learning to predict protein-protein interactions from protein sequences. *Bioinformatics*.**19(15)**:1875-1881.

Han, D.S., Kim,H.S., Jang, W.H. and Lee, S.D. (2004). PreSPI: A Domain Combination Based Prediction System for Protein-Protein Interaction. *Nucleic Acids Research.* **32(21):** 6312-6320.

Harrington, H.C., Rosenow, C. and Retief, J. (2000). Monitoring gene expression using DNA microarrays. *Curr. Opin. Microbiol.* **3**:285-291.

Heckerman, D., Geiger, D., and Chickering, D. (1995). Learning Bayesian networks: The combination of knowledge and statistical data. *Machine Learning*. **20**:197-243.

Henikoff, S. and Henikoff J.G., (1994). Protein family classification based on searching a database of blocks. *Genomics*. **19**:97-107.

Hermjakob, H., Montecchi-Palazzi, L., Bader, G., Wojcik, J., Salwinski, L., Ceol, A., Moore, S., Orchard, S., Sarkans, U., von Mering, C., *et al*. (2004). The HUPO PSI's Molecular Interaction format - A community standard for the representation of protein interaction data. *Nature Biotechnology*. **22**:177-183.

Hishigaki, H., Nakai, K., Ono, T., Tanigami, A. and Takagi, T. (2001). Assessment of prediction accuracy of protein function from protein-protein interaction data. *Yeast*. **18**:523-531.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S., Millar, A., Taylor, P., Bennett, K., Boutilier, K. et al. (2002). Systematic identification of

protein complexes in Saccharomyces cerevisiae by mass spectrometry. *Nature*. **415:**180 183.

Hodgman, T. C. (2000). A historical perspective on gene/protein functional assignment. *Bioinformatics*. **16**:10-15

Hong, E.L., Balakrishnan, R., Christie, K.R., Costanzo, M.C., Dwight, S.S., Engel, S.R., Fisk, D.G., Hirschman, J.E., Livstone, M.S., Nash, R., Oughtred, R., Park, J., *et al*., (2005). Saccharomyces Genome Database. http://www.yeastgenome.org/ (16/2/2005).

Hopp, T.P. and Woods, K.R. (1981). Predicting of protein antigenic determinants from amino acid sequences. *Proc. Natl Acad. Sci. USA.* 7 8(6): 3824-3828 .

Hsu, C.W. and Lin, C. J. (2002). A simple decomposition method for support vector machines. *Machine Learning*. **46**:291-314**.**

Huang, C., Morcos, F., Kanaan, S.P., Wuchty, S., Chen, D.Z., and Izaguirre, J.A. (2007). Predicting Protein-Protein Interactions from Protein Domains Using a Set Cover Approach. *EEE/ACM Transactions on Computational Biology and Bioinformatics* (TCBB). 4(1):78-87.

Huang, Y., Frishman, D., Muchnik, I. (2004). Predicting Protein-Protein Interactions by a Supervised Learning Classifier. Computational Biology and Chemistry , 28, 4, 291-301.

Ito, T., Tashiro, K., Muta, S., Ozawa, R., Chiba, T., Nishizawa, M., Yamamoto, K., Kuhara, S. and Sakaki, Y. (2000). Toward a protein-protein interaction map of the budding yeast: a comprehensive system to examine two-hybrid interactions in all possible combinations between the yeast proteins. *Proc. Natl. Acad. Sci*. USA. **97**: 1143-1147.

Jansen, R., Yu, H., Greenbaum, D., Kluger,Y., Krogan, N.J., Chung, S., Emili, A., Snyder, M., Greenblatt, J.F. and Gerstein, M. (2003). A Bayesian networks approach for predicting protein-protein interactions from genomic data. *Science*. **302**: 449-53.

Japkowicz, N. (1999). *Concept-Learning in the absence of counterexamples: an autoassociation-based approach to classification*. PhD thesis, New Brunswick Rutgers, The State University of New Jersey**.**

Jensen, F. (1996). An Introduction to Bayesian Networks. *UCL Press*, London.

Jones, S. and Thornton J. M. (1997). Prediction of protein-protein interaction sites using patch analysis. *J. Mol. Biol.* **272**:133-143.

Karplus, K., Kimmen, S., Barrett, C., Cline, M., Haussler, D., Hughey, R., Holm, L. and Sander, C. (1997). Predicting protein structure using hidden Markov models. *Proteins: Structure, Function, and Genetics*, **Suppl. 1**:134-139.

Keerthi, S.S. and C.J. Lin (2003). Asymptotic behaviors of support vector machines with Gaussian kernel. *Neural Computation*. **15(7)**:1667–1689**.**

Kim, W.K., Park, J., and Suh, J.K. (2002). Large scale statistical prediction of protein-protein interaction by potentially interacting domain (PID) pair. *Genome Informatics*. **13**:42-50.

King, R.D., Karwath, A., Clare, A. and Dehaspe, L. (2001). The utility of different representations of protein sequence for predicting functional class. *Bioinformatics*. **17:** 445-454.

Kini, R.M. and Evans, H.J. (1996). Prediction of potential protein-protein interaction sites from amino acid sequence identification of a fibrin polymerization site. *FEBS letters*. **385:** 81-86.

Koike, A. and Takagi, T. (2003). Prediction of Protein Interaction Sites and Protein-Protein Interaction Pairs Using Support Vector Machines. *Genome Informatics*. **14**:500-501.

Koller, D. (1999). Probabilistic Relational Models Source. *Lecture Notes In Computer Science*. **1634**: 3 - 13

Korf, I., Yandell, M., and Bedell, J. (2003). BLAST: Basic Local Alignment Search Tool. O'Reilly & Associates.

Kuhn, H. W. and Tucker, A. W. (1951). Nonlinear programming. *In Proceedings of 2nd Berkeley Symposium on Mathematical Statistics and Probabilistic*. Berkeley, University of California:481-492**.**

Larrañaga,P., Gallego, M.Y. Sierra, B. Urkola,L., and Michelena, M.J. (1997). Bayesian networks, rule induction and logistic regression in the prediction of the survival of women suffering from breast cancer. *Lecture Notes in Artificial Intelligence*. **1323**. E. Costa, A. Cardoso (eds.):303-308. Springer-Verlag.

Legrain, P., Wojcik, J., and Gauthier, J. (2001) Protein-protein interaction maps: a lead towards cellular functions. *Trends in Genet*. **17:**346-352.

Letovsky, S., and Kasif, S. (2003). Predicting protein function from protein-protein interaction data: a probabilistic approach. *Bioinformatics* **Vol. 19 Suppl. 1:**i197–i204.

Lichtarge, O., Bourne, H.R. and Cohen, F.E. (1996). An evolutionary trace method defines binding surfaces common to protein families. *J. Mol. Biol.* **257**:342-358.

Lin, N., Wu, B., Jansen, R., Gerstein, M., and Zhao H. (2004). Information assessment on predicting protein-protein interactions. *BMC Bioinformatics*. **5**:154.

Lodish, H., Berk, A., Matsudaira, P., Kaiser, C.A., Krieger, M., Scott, M.P., Zipurksy, S.L., and Darnell, J. (2004). Molecular Cell Biology 5th ed. WH Freeman and Company: New York, NY.

Lu, L., Lu, H., and Skolnick, J. (2002). MULTIPROSPECTOR: An algorithm for the prediction of protein-protein interactions by multimeric threading. *Proteins*. **49**: 350-364.

Marcotte, E.M., Pellegrini, M., Ng, H., Rice, D.W., Yeates, T.O., and Eisenberg, D. (1999). Detecting Protein Function and Protein-Protein Interactions from Genome Sequences. *Science.* **285(5428)**:751–753.

Marcotte, E.M., Xenarios, I., van Der Bliek, A.M. and Eisenberg, D. (2000). Localizing proteins in the cell from their phylogenetic profiles, *Proc. Natl. Acad. Sci. USA* **97**:12,115–12,120

Moya, M., Koch, M., and Hostetler, L. (1993). One-class classifier networks for target recognition applications. *In Proceedings world congress on neural networks*. Portland, OR. International Neural Network Society, INNS:797–801**.**

Mulder, N.J., Apweiler, R., Attwood, T. K., Bairoch, A., Barrell, D., Bateman, A., Binns, D., *et al*., (2003). The InterPro Database brings increased coverage and new features. *Nucleic Acids Research*. **31**:315-318.

Müller, K. R., Mika, S., Ratsch, G., Tsuda, K., & Schölkopf, B. (2001). An introduction to kernel-based learning algorithms. *IEEE Transactions on Neural Networks*. **12(2)**:181–201.

Newman, J. R., Wolf, E., and Kim, P. S. (2000). A computationally directed screen identifying interacting coiled coils from Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. U.S.A.* **97:**13203–13208

Ng, S., Zhang, Z. and Tan, S. (2003). Integrative approach for computationally inferring protein domaininteractions. *Bioinformatics*. **19**:923-929.

Niculescu, R., and Mitchell, T. (2006). Bayesian network learning with parameter constraints. *Journal of Machine Learning Research*. **7**:1357–1383.

Norin, M. and Sundstrom, M. (2002). Structural proteomics: developments in structure-to-function predictions. *Trends in Biotechnology*. **20**:79-84.

Ofran, Y. and Rost, B. (2003). Predict protein–protein interaction sites from local sequence information. *FEBS Letters*. **544**:236–239.

Osuna, E., Freund, R., and Girosi, F. (1997). An improved training algorithm for support vector machines. *In Proc of IEEE NNSP'97*. Amelia Island:24-26.

Oyama, T., Kitano, K., Satou, K. and Ito, T. (2000). Mining association rules related to protein-protein interactions. *Genome Informatics*. **11**:358–359.

Palsson, B. (2000). The challenges of in silico biology. *Nature Biotechnology*. **18**:1147-1150.

Pawson, T. and Nash, P. (2003). Assembly of cell regulatory systems through protein interaction domains. *Science*. **300**:445–452.

Pazos, F., Helmer-Citterich, M., Ausiello, G. and Valencia, A. (1997). Correlated mutations contain information about protein-protein interaction. *J. Mol. Biol.* **271**:511-523.

Pearson,W.R. (2000). Flexible sequence similarity searching with the FASTA3 program package. *Methods Mol. Biol*. **132**:185-219.

Pellegrini, M., Marcotte, E., Thompsom, M.J., Eisemberg, D. and Yeates, T.O. (1999). Assigning protein functions by comparative genome analysis: Protein phylogenetic profiles. *Proc. Nat. Acad. Sci*. **96**:4285-4288.

Phizicky, E.M. and Fields, S. (1995). Protein-Protein Interactions: Methods for Detection and Analysis. *Microbiological Reviews* **(Mar.)**:94-123.

Punta, M. and Rost, B. (2005). PROFcon: novel prediction of long-range contacts. *Bioinformatics*. **21**:2960–2968.

Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. (1999). A generic protein purification method for protein complex characterization and proteome exploration**.** *Nature Biotechnology*. **17(10)**:1030-1032**.**

Riley, R., Lee, C., Sabatti, C., and Eisenberg , D. (2005). Inferring protein domain interactions from databases of interacting proteins. *Genome Biology*. **6**:R89

Ritter, G. and Gallegos, M. (1997). Outliers in statistical pattern recognition and an application to automatic chromosome classification. *Pattern Recognition Letters*, **18**:525–539**.**

Salamov, A.A. and Solovyev, V.V. (1995). Prediction of protein secondary structure by combining nearest- neighbor algorithms and multiple sequence alignments. *J. Mol. Biol.* **247**:11-15.

Salwinski, L. and Eisenberg, D. (2003) Computational methods of analysis of protein–protein interactions. *Curr. Opin. Struct. Biol*. **13**:377–382.

Schölkopf, B. Smola, A., and Muller K.R. (1999). *Kernel principal component analysis*. MIT Press.

Schölkopf, B., and Smola, A. (2002). *Learning with kernels—support vector machines, regularization, optimization and beyond*. Cambridge, MA: MIT Press.

Schwikowski, B., Uetz, P. and Fields, S. (2000). A network of protein-protein interactions in yeast. *Nature Biotechnology* **18**:1257-1261.

Selbach, M. and Mann, M. (2006). Protein interaction screening by quantitative immunoprecipitation combined with knockdown (QUICK). *Nature Methods*. **3**:981-983.

Shawe-Taylor, J. and Cristianini, N. (2000). *An Introduction to Support Vector Machines and Other Kernel-based Learning Methods*. Cambridge University Press.

Shawe-Taylor, J. and Cristianini, N. (2004). *Kernel Methods for Pattern Analysis*. Cambridge University Press.

Shen, J., Zhang, J., Luo, X., Zhu, W., Yu, K., Chen, K., Li, Y., and Jiang, H. (2007). Predicting protein–protein interactions based only on sequences information. *PNAS*. **104**:4337-4341.

Shin, H.J., Eom D.H. and Kim S.S. (2005). One-class support vector machines: an application in machine fault detection and classification. *Computers and Industrial Engineering*. **48(2)**:395–408.

Sikora, M., Morcos, F., Costello, D.J., and Izaguirre J.A. (2007). Bayesian Inference of Protein and Domain Interactions Using the Sum-Product Algorithm. *Proc. Information Theory and Applications Workshop*, San Diego, Jan. 29**.**

Sonnhammer, E.L.L. and Kahn, D. (1994). Modular arrangement of proteins as inferred from analysis of homology. *Protein Science.* **3**:482-492.

Tipping, M. (1999). The relevance vector machine. *In Advances in Neural Information Processing Systems*. **12**.

Tong, A.H, Drees, B., Nardelli, G., Bader G.D., Brannetti, B., Castagnoli, L., Evangelista, M., Ferracuti, S., Nelson, B., Paoluzi, S., et al. (2002). A combined

experimental and computational strategy to define protein interaction networks for peptide recognition modules. *Science*. **295**:321-324.

Tong, A.H., Evangelista, M., Parsons, A.B., Xu, H., Bader, G.D., Page, N., *et al*. (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science*. **294:**2364–2368.

Uetz, P. and Vollert, C.S. (2005). Protein-Protein Interactions. *Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine* (ERGPMM), Springer Verlag. **16**:1548-1552.

Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, *et al*. (2000). A Comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. *Nature* **403**:623 627.

Vapnik V.N. (1995). *The Nature of Statistical Learning Theory*. Springer.

Vazquez A., Flammini, A., Maritan, A., and Vespignani, A. (2003). Global protein function prediction in protein-protein interaction networks. *Nature Biothech.* **21**:697-702.

Vert, J.P., Tsuda, K., and Schölkopf, B. (2004). *A Primer on Kernel Methods In: Kernel Methods in Computational Biology*. MIT Press.

Voet, D. and Voet, J.G. (2004). *Biochemistry*. Vol 1 3rd ed. Wiley: Hoboken, NJ.

von Mering, C., Krause, R., Snel, B., Cornell, M., Oliver, S.G., Fields, S., and Bork, P. (2002). Comparative assessment of large-scale data sets of protein–protein interactions. *Nature*. **417(6887)**:399–403.

Walhout, A. and Vidal, M. (2001). Protein interaction maps for model organisms. *Nature Reviews Molecular Cell Biology*. **2**:55-62.

Walhout, A.J., Reboul, J., Shtanko, O., Bertin, N., Vaglio, P., Ge, H., Lee, H., Doucette-Stamm, L., Gunsalus, K.C., Schetter, A.J., Morton, D.G., Kemphues, K.J., Reinke, V., Kim, S.K., Piano, F., Vidal, M., (2002). Integrating interactome, phenome, and transcriptome mapping data for the *C. elegans* germline. *Current Biology*. **12**:1952–1958.

Wojcik, J. and Schachter, V. (2001) Protein-protein interaction map inference using interacting domain profile pairs. *Bioinformatics*. **17**:296–305.

Wu, X., Zhu, L., Guo, J., Zhang D., and Lin K. (2006). Prediction of yeast protein–protein interaction network: insights from the Gene Ontology and annotations. *Nucleic Acids Research*. **34(7)**:2137-2150.

Xenarios, I., Salwinski, L., Duan, X.J., Higney, P., Kim, S.M., and Eisenberg, D., (2002). DIP: the Database of Interacting Proteins: a research tool for studying cellular networks of protein interactions. *Nucl. Acids. Res.* **30(1)**:303- 305.

Young, L., Jernigan, R.L., and Covell, D.G. (1994). A role for surface hydrophobicity in protein-protein recognition. *Protein Science*. **3**:717–729.

Yu, J., Fotouhi, F., and Finley, R.L. (2005). Combining Bayesian Networks and Decision Trees to Predict Drosophila melanogaster Protein-Protein Interactions. *In the 21st International Conference on Data Engineering Workshops*. April 5-8. Tokyo, Japan.

Zhou, H.X. and Shan, Y. (2001). Prediction of protein interaction sites from sequence profile and residue neighbor list**.** *Proteins*. **44(3)**:336-343.

Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., *et al*. (2001). Global analysis of protein activities using proteome chips. *Science*. **293**:2101-2105.