# Deriving Protein Networks by Combining Gene Expression and Protein Chip Analysis

**(HS-IDA-MD-02-202)**

**Ida Gunnarsson (a98idagu@student.his.se)**

*Institutionen för datavetenskap*
*Högskolan i Skövde, Box 408*
*S-54128 Skövde, SWEDEN*

Magisteravhandling inom Datavetenskap, våren 2002

Program för bioinformatik

Handledare: Angelica Lindlöf, Jesper Tegnér

# Deriving Protein Networks by Combining Gene Expression and Protein Chip Analysis

Submitted by Ida Gunnarsson to Högskolan Skövde as a dissertation for the degree of M.Sc., in the Department of Computer Science.

**[2002-06-07]**

I certify that all material in this dissertation which is not my own work has been identified and that no material is included for which a degree has previously been conferred on me.

Signed: _______________________________________________

# Deriving Protein Networks by Combining Gene Expression and Protein Chip Analysis

**Ida Gunnarsson (a98idagu@student.his.se)**

# Abstract

In order to derive reliable protein networks it has recently been suggested that the combination of information from both gene and protein level is required. In this thesis a combination of gene expression and protein chip analysis was performed when constructing protein networks. Proteins with high affinity to the same substrates and encoded by genes with high correlation is here thought to constitute reliable protein networks. The protein networks derived are unfortunately not as reliable as were hoped for. According to the tests performed, the method derived in this thesis does not perform more than slightly better than chance. However, the poor results can depend on the data used, since mismatching and shortage of data has been evident.

**Keywords:** Protein networks, protein chips, gene expression analysis

# Table of contents

1 Introduction....................................................................1

2 Background ....................................................................5

2.1 Biological networks ...................................................................... 6

2.2 Protein networks ........................................................................... 7

2.3 Gene expression technique.......................................................... 10

2.3 Protein chip technique.................................................................. 12

3 Related work..................................................................15

3.1 Derivation of protein networks.................................................... 15

3.2 Gene expression analysis............................................................. 16

3.3 Protein chip analysis..................................................................... 17

3.5 Relevance of related work............................................................ 19

4 Problem statement ........................................................20

4.1 Problem definition........................................................................ 20

4.2 Hypothesis..................................................................................... 21

4.3 Aims and objectives...................................................................... 22

4.4 Motivation...................................................................................... 23

4.5 Limitations..................................................................................... 24

5 Method............................................................................26

5.1 Course of action............................................................................. 26

5.1.1 Obtaining suitable data.............................................................. 26

5.1.2 Extraction of genes from gene expression data encoding proteins on chip............................................................................................ 28

5.1.3 Investigate the correlation between genes whose proteins interact on a protein chip ............................................................................. 28

5.1.4 Derivation of protein network .................................................. 29

5.1.5 Evaluation of derived protein network ..................................... 29

5.2 Experimental data ......................................................................... 30

5.2.1 Gene expression data ................................................................. 30

5.2.2 Protein chip data......................................................................... 32

5.2.3 Protein network .......................................................................... 33

5.3 Correlation measurement .............................................................. 33

5.4 Experiments.................................................................................... 35

5.5 Method for evaluation.................................................................... 38

**6 Results and analysis ........ 40**
**6.1 Experiment 1** ........ 40
**6.2 Experiment 2** ........ 45
**6.3 Experiment 3** ........ 50
**6.3.1 Experiment 3a** ........ 50
**6.3.2 Experiment 3b** ........ 53
**7 Discussion ........ 56**
**7.1 Future work** ........ 59
**8 Conclusions ........ 62**
**References ........ 63**
**Appendix ........ 68**

# 1 Introduction

The characterization of several entire genomes has resulted in the discovery of many new genes whose functions are yet unknown. For example, in yeast the function of nearly a third of all genes have not yet been determined (Snyder *et al.,* 2002; Uetz *et al.,* 2000). In an attempt to reveal their function and regulation, the use of gene expression analysis through microarrays has become a common and effective way of studying genes. One of the most important uses of microarrays is to study changes in gene expression that accompany changes in cell physiology, such as during development, cell-cycle progression, drug treatment or disease progression (He and Friend, 2001). A number of studies over the last few years have implied that the cellular state can be characterized and classified by gene expression patterns (He and Friend, 2001).

A great amount of information about gene function comes from the analysis of the biochemical activities of the encoded protein (Zhu *et al*., 2000). Therefore, research has been directing towards the proteomic field, i.e. the identification and characterization of proteins. There are several different methods to perform studies on protein level, for example two-hybrid interactions (Uetz *et al.,* 2000), protein chips (Zhu *et al.,* 2000; MacBeath and Schreiber, 2000; Zhu *et al*., 2001), mass spectrometry (Gavin *et al.,* 2002; Ho *et al.,* 2002) and protein network development (Schwikowski *et al.,* 2000; D'haeseleer and Fuhrman, 1999).

The absence of a direct functional correlation between gene transcripts and their corresponding proteins, however, represents a significant obstacle for improving the efficiency of drug discovery (Hazbun and Fields, 2001). The expression arrays allow us to monitor thousands of individual genes in a single experiment. In addition to the classification of disease specimens, this technology has led to a broad spectrum of applications such as drug target validation, pathway dissection, discovery of gene function and annotation of the human genome (He and Friend, 2001). The correlation between mRNA level and protein abundance is not direct, it is in this case more valuable to measure the protein activity. The availability of an entire genome sequence makes it possible to perform biochemical assays on every protein encoded by the genome (Zhu *et al.*, 2000). In addition, using protein microarrays, hundreds or thousands of protein samples can be analysed in parallel (Zhu *et al.*, 2001).

Fully sequenced genomes lead to additional insights into the functional properties of the encoded proteins. These functional insights emerge as networks of interacting proteins (Xenarios and Eisenberg, 2001). Networks provide testable hypotheses, which eventually would improve our understanding of the cells molecular machinery. Such hypothetical networks or protein complexes would also serve as the appropriate targets for proteomics-based analysis (Ito *et al.*, 2001) and functional genomic analysis (Xenarios and Eisenberg, 2001).

This thesis deals with the area of protein networks, which is defined by the interactions that exist between different proteins in an organism. Protein networks point out global patterns of interactions among proteins. The proteins constituting a network belong to the same protein complex or have the same cellular localization (Schwikowski *et al.*, 2000). Networks can be derived in several different manners, but one important aspect that recently has been placed emphasis on, is the combination of different approaches in order to increase reliability (Hatzimanikatis and Lee, 1999).

Gene expression analysis and protein-protein interactions have previously been used individually to form different networks. Protein chips are a relatively new form of studying the expression of several proteins at one time. This approach is the counterpart for proteins that microarrays are for genes.

In this thesis gene expression and protein chip analysis are used in combination for the derivation of reliable protein networks. **The hypothesis stated is that protein networks can be derived from the combination of gene expression analysis and protein chip data.** The hypothesis is verified when the derived protein network agrees with the existing network used for validation. The hypothesis is on the other hand falsified if there is evidence that the derived protein network do not agree with a reliable existing network.

Co-expressed genes or proteins that are found in the same protein complex or in the same location are likely to be involved in the same or related cellular process (Hazbun and Fields, 2001). Consequently, genes encoding these proteins would be expected to be co-expressed. Co-expressed genes have the same expression profiles over different time-points but need not to necessarily share the same regulation (D´haeseleer *et al.*, 2000).

In chapter 2 the concepts and definitions of protein networks, protein chips and gene expression analysis are being brought up. In chapter 3 related works being made in the areas of interest are considered. Problem definition, hypothesis, aims and objectives are described together with the motivation behind this thesis in chapter 4. Chapter 5 contains the method, where there is to be found a description of the course of action, 5.1, together with a survey of the experimental data used in the experiments, 5.2. The chapter continues with a description of the correlation measurement, 5.3, and the method used for evaluation, 5.4. Finally, a thorough review of the experiments and how they are performed completes this chapter. Results and analysis of the different experiments are depicted in chapter 6, and a discussion about the method is found in the final chapter 7, together with a section (7.1) discussing future work. The entire thesis is brought to an end in chapter 8, where conclusions are being summarized.

# 2 Background

In recent years the focus has shifted from comparative analysis of gene sequences and the sequencing of genomes to the analysis of function and regulation of genes and their encoding proteins. Proteins are the final products of genes where one gene is responsible for the production of a single polypeptide chain (Weaver and Hedrick, 1997). Each protein consists of one or several polypeptide chains and its function is determined by the proteins unique three-dimensional conformation. Transportation of substances, structural support, protection against diseases and catalyzing chemical reactions as enzymes or hormones are a few of the functions carried out by different proteins (Campbell *et al*., 1999).

The process in which proteins are made out of genes is known as the central dogma and is carried out in two major steps, known as transcription and translation (Figure 1). The transcription phase involves the construction of an RNA copy of a gene, a messenger RNA (mRNA). The information in this mRNA is then used to create a protein and this phase is the translation step. Translation involves the conversion of the nucleotide sequence of an mRNA to the amino acid sequence that constitutes the protein. After the translation most proteins undergo a post-translational modification in order to fold properly.

All higher organisms consist of large amounts of genes and proteins. The genes and proteins interact with each other in many different cellular processes. Biological networks are a way of compiling these interactions.

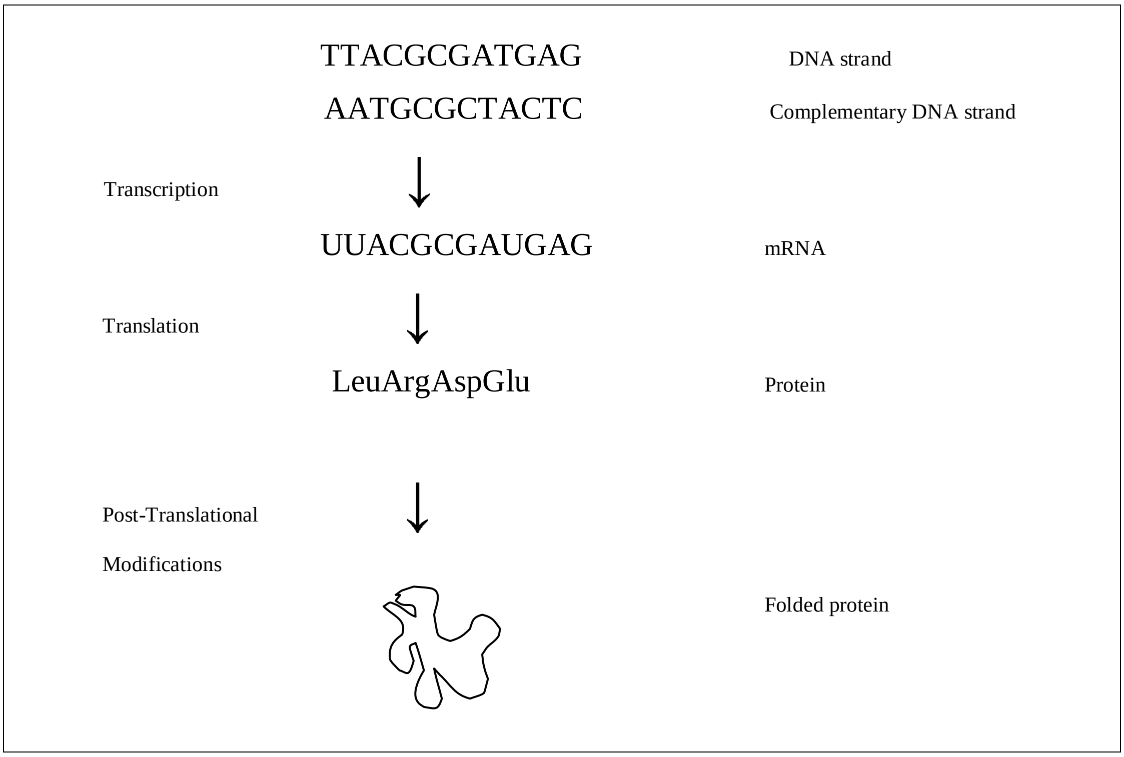


**Figure 1**. A summary of the conversion from gene to protein.

## 2.1 Biological networks

A network can be viewed as a set of subunits that interact with each other. The subunits can be either genes or proteins and the links between these genes or proteins describe the connections. Cellular processes are controlled by various types of biological networks. For example, a metabolic network controls the processes which generate mass and energy from nutritive matter. The nodes in such a network are the substrates. Two substrates are connected by a link if both of them participate in the same biochemical reaction.

In a protein-protein interaction network, the nodes represent the proteins. A link exists between two nodes if the corresponding proteins have a direct physical interaction. Protein networks are discussed in greater detail in Section 2.2.

A metabolic network is one of several different types of cellular networks, other types are the cell cycle network and the apoptosis network (Jeong *et al.*, 2000). Metabolic networks differ in some way in their definition from protein networks. A metabolic network is described as a collection of enzymatic reactions that serve to biochemically process metabolites within the cell and transport processes that convert extra cellular metabolites to intracellular metabolites and vice versa (Edwards and Palsson, 2000). A metabolic network is built up of nodes, the substrates, which are connected to one another through links, which are the actual metabolic reactions. The physical entity of the link is the temporary educt-educt complex itself, in which enzymes provide the catalytic scaffolds for the reactions yielding products, which in turn can become educts for subsequent reactions (Jeong *et al.*, 2000). The protein network is, as already mentioned, a description of the interactions among proteins. The protein network provides only protein interactions, whereas the metabolic network consists of a more detailed description. The metabolic networks prove to have the same properties as the protein networks, i.e. they show a scale-free appearance, and are tolerant to random errors (Jeong *et al.*, 2000). The construction of metabolic networks is a demanding, small-scale method that can be very time-consuming (Edwards and Palsson, 2000; Jeong *et al.*, 2000).

## 2.2 Protein networks

A protein network is a visualisation of the interactions that exist between different proteins in an organism, and is defined by the sum of interactions. Networks and the accompanying computational approaches can be used to view global patterns of interactions among proteins belonging to the same protein complex or having the same cellular localization (Schwikowski *et al*., 2000). In higher metazoa, each gene or protein is estimated on average to interact with four to eight other genes and to be involved in ten biological functions (D´haeseleer *et al.*, 2000).

Proteins can have direct or indirect interactions with one another. Direct interactions refer to two proteins that bind to each other. Indirect interaction on the other hand refers to being a member of the same functional module (e.g., transcription initiation complex, ribosome etc.). Proteins belonging to the same module are not necessarily directly bond to one another (Jeong *et al.*, 2001b). The environment or developmental status of the cell affects the presence or absence of the interactions; hence these interactions reflect the dynamic state of the cell.

A general network is either exponential or scale-free. In an exponential network all the nodes are connected with about the same number of nodes, unlike scale-free networks. The scale-free network consists of a small number of nodes that are connected to several other nodes and remaining nodes are only connected to one or a few other nodes (Figure 2). Protein networks are extremely heterogeneous, in difference from exponential networks, and are commonly found to be scale-free. This means that a few highly connected proteins link the rest of the less connected proteins to the system (Jeong *et al*., 2001a). This inhomogeneous structure contributes to the network's tolerance to random errors simultaneously to its sensitivity to loss of the most connected proteins (Jeong *et al*., 2001a). Highly connected proteins with a central role in the networks architecture are three times more likely to be essential than proteins with only a small number of links to other proteins (Jeong *et al*., 2001b).

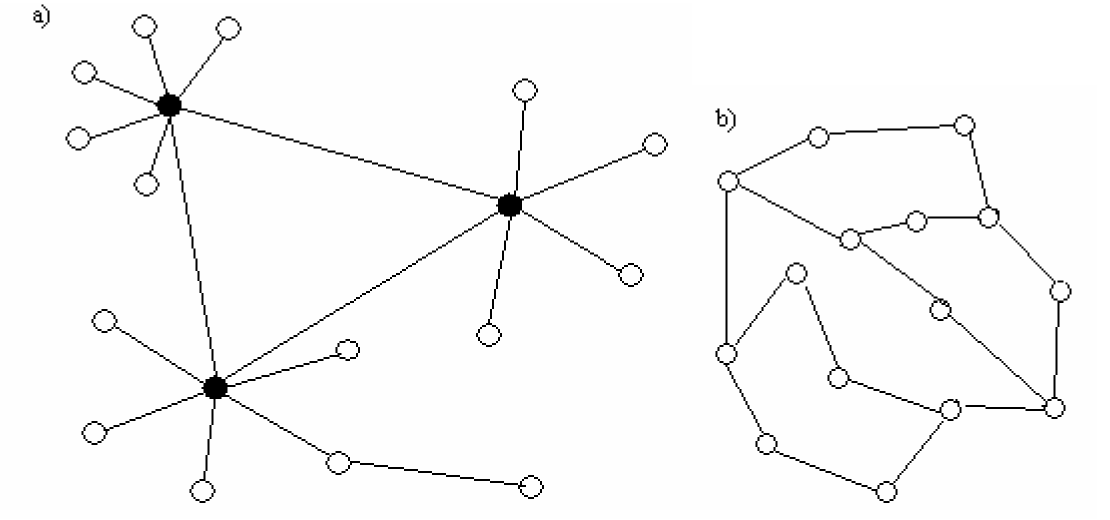


**Figure 2.** Networks where circles represent proteins and edges links between proteins. a) demonstrates a scale-free network, where most nodes have only a few edges, but a few nodes have a very large number of edges (black circles). b) is an exponential network, where all proteins is connected to about the same number of other proteins.

Furthermore, proteins with higher connectivity might have a higher probability of being lethal (Jeong *et al*., 2001b).

The links between proteins in the network can either be directed or undirected. A network is directed when one protein is known to affect another protein but not the other way around. If protein A affects protein B the connection is directed from A towards B. In an undirected network the connections are not known to affect each other in a specific manner. See Figure 3 for an illustration of directed and undirected protein interactions. When the functions of proteins linked together are known, it is possible to establish the directionality of the interaction, i.e. if there is a direction. Even though the directions are not established it is feasible to uncover the structure of the network (Jeong *et al*., 2001b).

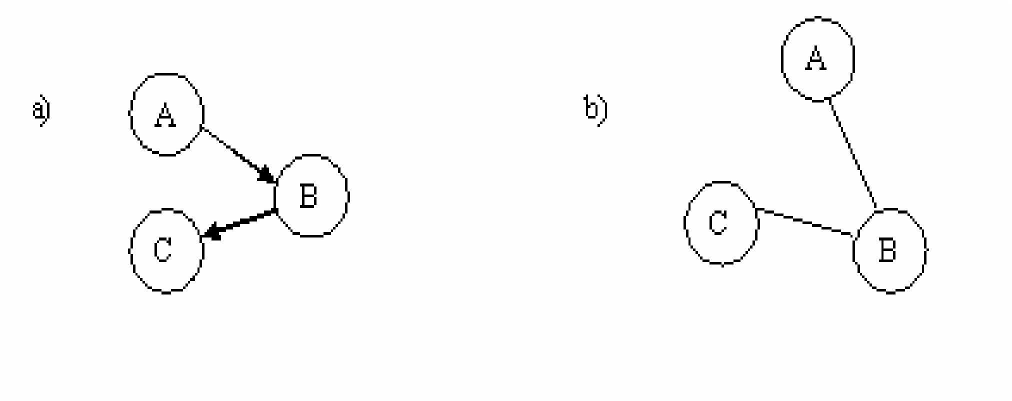


**Figure 3.** Two different types of protein networks represented by circles and edges. In a) a directed protein network, where a circle is a protein and the directed edges represents how a protein affects another protein. A affects B, which in turn affects C. In b) an undirected protein network. It is possible to detect that A and B affects each other, but not how. B and C are also affecting each other in some undefined way.

## 2.3 Gene expression technique

Since several eukaryote genomes are known, the interest has been turned towards using these known genome sequences to understand how the genomes function (Brazma and Vilo, 2000). Of special interest is, among other things, the functional role of different genes and in which cellular processes they participate, how genes are regulated and how they interact with each other and other gene products. Other intriguing issues are how the expression patterns differentiate in different types of cells, during different conditions, and how diseases and treatments affect these patterns. This is for example of great significance in drug discovery (Fannon, 1996; D´haeseleer *et al.*, 1999).

By using advanced techniques it has now become feasible to measure the expression levels for most, and sometimes all of the genes in an organism simultaneously. Hopefully this will enable the reconstruction of gene regulatory networks and hence result in greater insight in how genes are co-regulated, how they regulate other genes and what their functions are (D´haeseleer *et al*., 1999). Gene expression analysis is an important method for deriving information about the amount of transcribed genes in different tissues, developmental stages and different conditions for cells. Different techniques have quickly been developed allowing an efficient measure of the abundance of an organisms transcribed genes (Brazma and Vilo, 2000).

DNA microarrays are one of the later breakthroughs in experimental biology, measuring the expressions for thousands of genes in a single experiment (Figure 4). A microarray is a glass surface where single stranded DNA-molecules (cDNA) are attached on specific spots. On each glass surface there are tens of thousands of spots and each spot is related to a single gene. The microarray takes advantage of the fact that single stranded DNA easily bind to a complementary mRNA sequence. The glass surface is washed with fluorescent mRNA, which binds to the cDNA on the surface. The magnitude of fluorescence is measured and the expression level for the participating genes can be determined. This is done during different conditions and in different tissues (Brazma and Vilo, 2000).

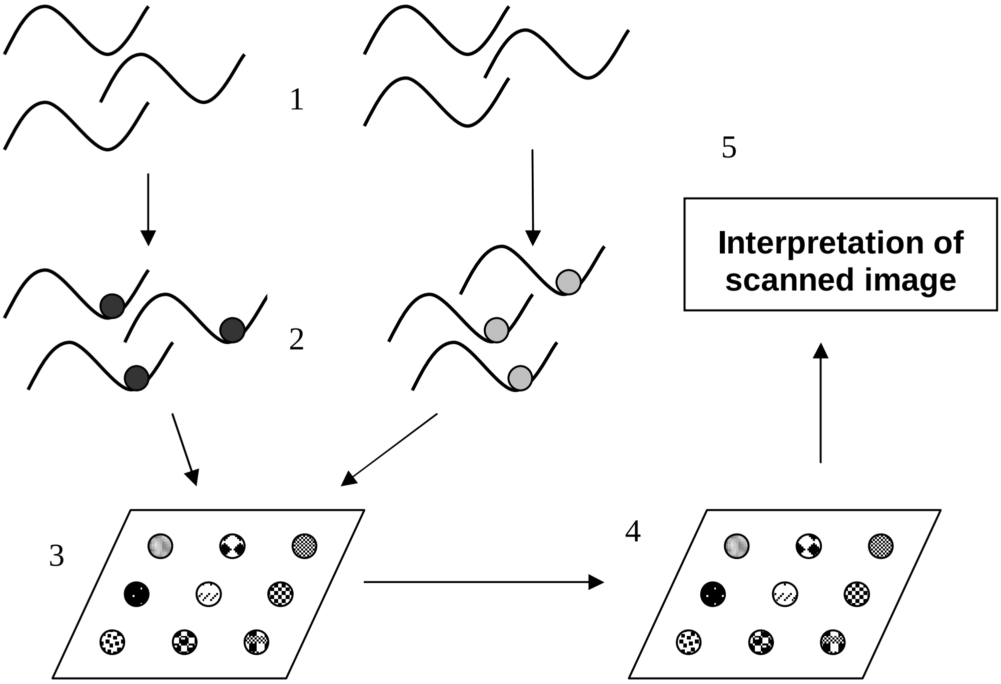


**Figure 4**. (1) Extracting mRNA molecules from the cell cultures and reverse transcribe them to cDNA’s. (2) Fluorescent labelling of cDNA’s. (3) Hybridization to a cDNA array. (4) Scanning the hybridized array. (5) Interpreting the scanned image.

## 2.3 Protein chip technique

Protein chips are a new technology to analyse protein activities. Using a protein chip it is feasible to analyse the functions of thousands of proteins in parallel. A protein chip is a microarray that is spotted with a substrate, e.g. specific proteins or molecules. The technique preserves the function of the proteins, in that the proteins attached are still able to interact with other proteins or molecules (Figure 5). This makes it possible to study protein-protein interactions, identifying the substrates of, for example, protein kinases and identifying the protein targets of small molecules (MacBeath and Schreiber, 2000). The chips marketed so far carry fewer than ten capture molecules, but a chip that could simultaneously analyse the production of tens of thousands of proteins is sought after, both from those engaged in fundamental research and from the drug industry (Abbott, 2002). The chips available today are suitable for certain applications, such as simple medical diagnostics. However, since the current chips manage to carry about a dozen proteins it would be possible to follow a number of proteins in a pathway and on this basis derive the whole pathway. On the other hand, for the large-scale proteomics projects that aim to determine how complex patterns of protein production vary with disease, they are presently inadequate (Abbott, 2002).

Earlier methods for studying interactions among proteins, like 2-hybrid screens and mass spectrometry, involve only two proteins at a time (Uetz *et al.,* 2000; Gavin *et al., 2002*). The most obvious advantage of the protein chip technology is that it enables the large-scale analysis of several different proteins towards the target protein, detected on the chip.

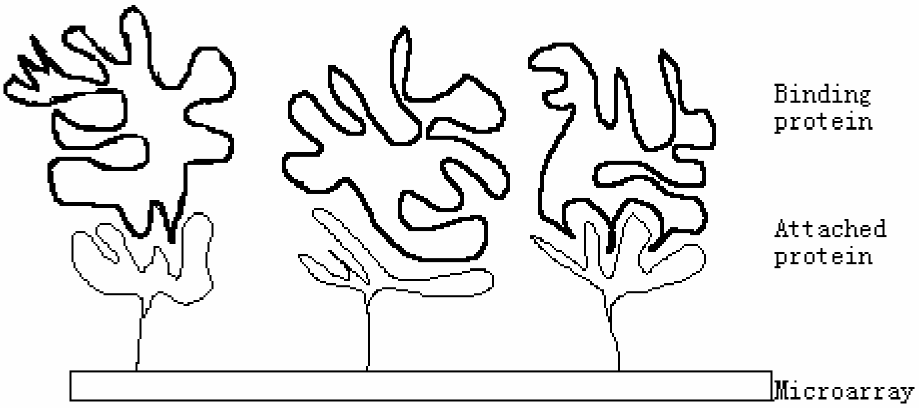


**Figure 5.** A protein chip with attached proteins. Free proteins are washed over the microarray and the proteins on the chip are able to interact with the free proteins.

Protein chip is a generic term for a number of different specialized chips that have been developed by different companies. Cambridge Antibody Technology is a company that attach antibodies to their chips, which is the most common way. The antibodies can be proteins, carbon hydrates or hormones and they have been chosen because they bind specifically to some substance of interest (Gustafsson, 2002). Finding a capture molecule that will bind with high affinity to one protein alone is extremely difficult. The capture molecules on a protein chip need to bind with high affinity because some of the most interesting proteins in a biological sample - such as hormones, growth factors and intracellular signalling proteins - are present only at very low concentrations (Abbott, 2002). The disadvantage of using antibodies is their tendency to be denatured when heated or exposed to other stresses. This means that they lose their structure and hence the ability to bind to other proteins.

Affibody is a company that uses another approach. They adopt a method where they develop special designed proteins, so called affibodies. These affibodies can find one single molecule among thousands of others in a sample and bind it.

# 3 Related work

A popular research area has been the development of protein networks. Several different approaches have been used and a few of them are described in the sections below. Gene expression analysis and protein chips have been used for not solely the purpose of deriving protein or gene networks, but these other applications are only mentioned briefly in this thesis.

As shown in sections below, both gene expression analysis, Section 3.2, and protein-protein interactions, Section 3.1, have been used for the purpose of constructing networks, but the use of expression of both genes and proteins in concert are still not explored. Nevertheless, there are indications given for the benefits of this combination, Section 3.4. Altogether, these related works assemble the separate aspects used in the hypothesis for this thesis.

## 3.1 Derivation of protein networks

Several methods for developing protein networks have been proposed. The analysis of protein complexes with mass spectrometry in order to form protein networks have been done by Gavin *et al.* (2002) and Ho *et al.* (2002). A two-hybrid interaction test has been performed by Tong *et al.* (2002) for the purpose of forming protein interaction networks.

Schwikowski *et al.* (2000) have constructed a network of 2 358 interactions among 1 548 proteins from *Saccharomyces cerevisiae.* The interactions between these proteins are detected using two-hybrid analysis. Interestingly enough, it has been found that proteins of known function and cellular location tend to cluster together and possible functions can be assigned to a protein based on the known functions of its interacting partners. This approach correctly predicts a functional category for 72% of the 1 393 characterized proteins with at least one partner of known function, and has predicted functions for 364 previously uncharacterized proteins.

## 3.2 Gene expression analysis

The abundance of information received with gene expression analysis demands powerful methods for deriving knowledge about the cell's expression profile (Eisen *et al.,* 1998). Several methods have been developed, amongst others different clustering algorithms. By clustering the expression profiles, patterns of similarity can be found in the data (Tamayo *et al.,* 1999). Since genes that share similar regulation and function supposedly have similar gene expression profiles, clustering algorithms are used for their ability to group these genes together (Eisen *et al.,* 1998; Zhu and Zhang, 2000; D'haeseleer *et al.,* 2000). Gene clustering tries to infer co-regulation and not the regulatory interactions between genes. For this purpose several other different approaches have been evolved (D'haeseleer *et al.,* 2000).

Reverse engineering is a method for extracting networks of regulatory interactions between genes from gene expression data (D'haeseleer *et al.,* 1999). Several algorithms have been developed for reverse engineering, for example, Liang *et al.* (1998) used Mutual Information for their procedure and Ideker *et al.* (2000) developed an algorithm using the branch and bound technique.

Another useful method to construct genetic networks from gene expression data by reverse engineering is by using the Boolean network approach. This approach radically simplifies the individual interactions between genes by representing each gene by a binary variable. A gene is either ON or OFF, representing the ON state when the mRNA level is above a certain threshold and OFF otherwise. When using Boolean networks the continuous values are exchanged to binary values, which are a major simplification of the genes representation (D'haeseleer *et al.*, 2000). The effect one gene has on another gene is represented by Boolean logical rules. Despite the simplifications made, the network is still very complex, and hence it is still difficult to derive interactions.

## 3.3 Protein chip analysis

Protein chips in general provide information about interactions between a vast amount of proteins with a specific target protein on the chip. See Section 2.3 for a detailed description.

Zhu *et al.* (2000) have developed a novel protein chip technology for analysis of biological activities and studied the protein kinases from *Saccharomyces cerevisiae.* 119 out of 122 known and predicted yeast protein kinases were overexpressed and analysed using 17 different substrates on different protein chips. This experiment resulted in the findings of several novel protein activities and that a large number of protein kinases are capable of phosphorylating tyrosine. In addition to these results Zhu *et al.* (2000) also identified a number of features of protein kinases not known before, as well as stated the usefulness of protein chip technology for high-throughput screening of protein biochemical activity.

Zhu *et al*., (2000) concluded that particular proteins are preferred substrates for particular protein kinases and vice versa. The experiments were performed for *in vitro* kinase activity and hence not ensured to be phosphorylated by the same kinases *in vivo*. The technology described in this article has some substantial advantages over conventional methods. The chip-based assays have for example very high signal-to-noise ratios and the amount of material needed is very small. Another advantage is the extreme sensitivity of the enzymatic assays the protein chips are using, and the inexpensiveness of the chips contributes as well to the favours of this approach.

MacBeath and Schreiber (2000) have developed miniaturized assays that accommodate extremely low sample volumes and enable the rapid, simultaneous processing of thousands of proteins. They spotted proteins onto chemically derivatized glass slides at extremely high spatial densities. The attached proteins yet retained their ability to interact specifically with other proteins. MacBeath and Schreiber demonstrated three different applications for the protein microarrays: screening for protein-protein interactions, identifying the substrates of protein kinases and identifying the protein targets of small molecules.

Zhu *et al*. (2001) have identified several new calmodulin- and phospholipid-interacting proteins by using protein microarrays. 5 800 open reading frames (ORFs) from the yeast proteome were screened for their ability to interact with proteins and phospholipids. A majority of the calmodulin-binding proteins were found to have a common potential binding motif. In addition to known partners, the calmodulin probe identified 33 additional potential partners. These include many different types of proteins, consistent with a role for calmodulin in many diverse cellular processes.

## 3.4 Mathematical modelling of gene networks

Hatzimanikatis and Lee (1999) argues that a combination of gene expression information at the message level and at the protein level is required to describe even simple models of gene networks. They tested whether gene expression data, either mRNA or protein, is sufficient to elucidate the relationship between genome sequence, gene regulation and cellular dynamics. This was done by studying and comparing two different mathematical modelling methods for genetic networks, Boolean and continuous. The result of this investigation was that gene regulation studies based on expression data or protein array data solely not withholds enough information and this can lead to incorrect conclusions about which genes are important to a particular phenotype.

## 3.5 Relevance of related work

Since there are no articles found that has done the same type of combination of protein chip and gene expression analysis made in this thesis, the related works considers only one aspect each of the derived method. These articles can be used in order to validate the results given in this thesis, or to point out different aspects that are not always making the results totally reliable. When combining different methods the possibility of circumvent these kind of ambiguities can be decreased.

# 4 Problem statement

This chapter introduces the problem, hypothesis and motivation behind this thesis. Section 4.1 constitutes the problem definition and Section 4.2 the hypothesis. A preliminary course of action is outlined together with the aims in Section 4.3 and finally the motivation behind this thesis is discussed in Section 4.4

## 4.1 Problem definition

Now when many genomes have been sequenced, the challenge is to study and understand the functions and regulations of the corresponding proteins (Zhu *et al.,* 2000; Xenarios and Eisenberg, 2001). There are several ways of studying the expression of both genes and proteins. For example, the gene expression analysis approach has become very popular for interpreting the co-expression of thousands of genes simultaneously. The gene expressions can be measured by using cDNA microarrays. It is generally assumed that genes with similar expression pattern are co-regulated and therefore presumably functionally related (D'haeseleer *et al.,* 2000). A new technology for analysis of protein activities is the use of protein chips. With the protein chip it is possible to study thousands of proteins in parallel (Zhu *et al.*, 2001) and makes it possible to study protein-protein interactions, identifying the substrates of for example protein kinases and identifying the protein targets of small molecules (MacBeath and Schreiber, 2001).

One limitation with the protein analysis approach at the moment is that it is not possible to draw any conclusions about which proteins correlate to each other. It is possible to find out which of the proteins that bind to the specific protein used on the chip, but the connection between all these proteins that bind to the chip protein is not possible to detect.

The information derived from gene expression experiments can be used for analysing genes encoding specific proteins. Investigations can be made whether a gene encoding a specific protein attached to the chip protein, has the same or similar expression pattern as the genes encoding the other proteins that bind to the chip protein. By doing this, there is a possibility to draw conclusions about the correlation between the proteins and hence find the protein network they belong to. Proteins that bind to the same chip protein and on the same time are encoded by genes with a high correlation, would together be able to constitute a reliable protein network.

## 4.2 Hypothesis

The hypothesis is that protein networks can be derived from the combination of gene expression analysis and protein chip data.

The proteins that bind to the same substrate are possibly located in the same area or members of the same protein complex. The hypothesis made is that if genes in an expression set are highly correlated, they could share regulation and hence be part of the same protein network. Thus, proteins adjacent in a network are expected to strongly correlate in their respective gene expressions. Therefore, combining data from protein chip and gene microarrays, it may be possible to propose new networks with genes that yet not have any known function.

Consequently, the hypothesis is verified when the derived protein network agrees with the existing network used for validation. The hypothesis is on the other hand falsified if there is evidence that the derived protein network do not agree with a reliable existing network.

If proteins highly correlated do not appear to be neighbours they could be situated close to each other in the protein network.

## 4.3 Aims and objectives

The aim for this thesis is to investigate if it is possible to derive protein networks from the combination of gene expression analysis and data extracted from protein chips. The derived network will be evaluated against a predefined protein network.

In order to achieve this aim, the following objectives need to be attained:

- Choose suitable gene expression data and protein chip data
- Choose a suitable known network for evaluation
- Develop a method for deriving protein networks
- Apply developed method on test data - four different experiments will be performed.
- Evaluate the performance of the method against the known network

In order to derive a protein network, genes will be extracted from the chosen gene expression set if they encode proteins that interacts with substrates attached to a chip in the protein data set. The next step will be to investigate if it is possible to derive a network on the basis of the protein chip analysis together with gene expression data, by the developed method. Four different experiments will be performed. The first two experiments are carried out with two different gene expression data sets. The last two will combine the data used in the first two experiments in order to find the best results. Finally, the derived protein network is evaluated against an existing network. If the produced protein network agrees with the existing network, it should be possible to derive new protein networks by using this method. These new networks could also contain genes with unknown function, which are not present in networks derived by using other methods. If this approach proves to produce reliable protein networks, the hypothesis is not to be falsified.

One limitation with the use of protein chips is, only proteins that bind directly to the chip protein will participate in the derived network. The possibility that other proteins, which do not directly bind to the chip protein, also would be part of the same protein network is evident.

Possible delimitations that may be necessary could be to reduce the amount of data used in the process of deriving a network. The protein data could be diminished as well as the gene expression data.

## 4.4 Motivation

The microarray technique has made it possible to effectively achieve large amounts of data about the expression level of genes. Despite many advantages there are although some limitations with this approach. One of the drawbacks is the absence of a direct correlation to the corresponding protein levels, since proteins are the final product and the usual target for drugs ( Brazma and Vilo, 2000; Hazbun and Fields, 2001; Ideker *et al.*, 2001). Instead of studying the gene expressions it is interesting to study protein levels. Protein chips are used for this purpose. It is a much more complex procedure and the techniques are not yet as effective as the gene level approach. Thus, the combination of these two different techniques takes into consideration the different advantages proposed by the different approaches.

Hatzimanikatis and Lee (1999) states that investigations of the regulation of genetic circuits based on quantitative mRNA expression levels alone neglect critical information, and argue for the need to combine data from mRNA expression levels and their protein counterparts to develop accurate descriptions of gene networks. Expression levels of many genes measured by mRNA analysis are significantly different from the levels measured with a proteomic strategy. Thus, the combination of these methods could improve reliability in predicting protein networks.

## 4.5 Limitations

In order to construct protein networks that are reliable and useful, all the proteins interacting with each other need to be participating in that network. With the approach used in this thesis, the collection of proteins restricts this possibility. This is because there is not, so far, enough information available with the protein chips, due to the amount of proteins on the chip is limited. Each chip only contain one protein that binds to other proteins that the chip is washed with, which means that whole protein networks is almost impossible to construct. The resulting interactions will instead form protein complexes, i.e. smaller subunits of the network.

Another limitation that can restrict the outcome is the similarity measure between the expression profiles of the encoding genes (Figure 6). One possibility is that desirable genes are not present in the gene expression data, which results in that the protein can not be used for this method. If the genes do exist in the data, they need to have similar expression patterns to be able to participate, otherwise they are disregarded. This can contribute to delimit the number of proteins that can be part of the derived complexes.

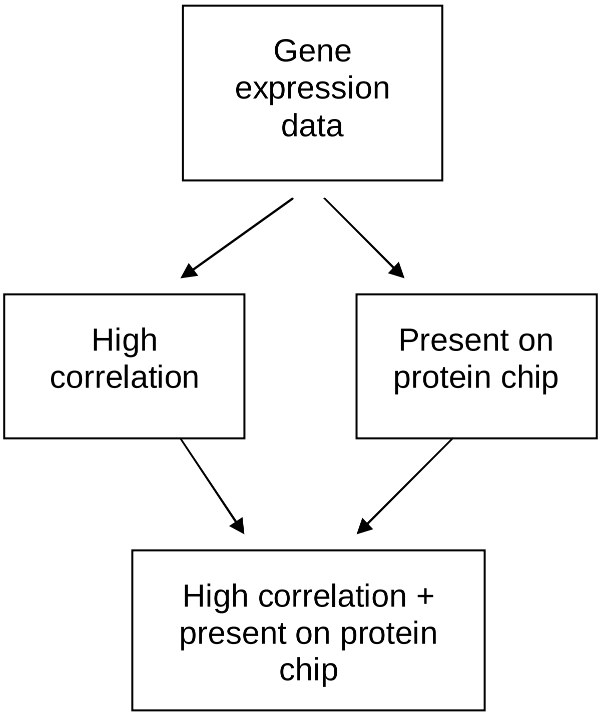


**Figure 6.** The genes found in the gene expression must have similar expression profiles and on the same time be present on the protein chip. This delimits the number of proteins that together is going to constitute the derived protein network.

# 5 Method

This chapter provides a description of the method developed in this thesis and how the aims and objectives are met. First, Section 5.1 a general description of the course of action is explained and thereafter Section 5.2, information about the different sources of data that have been used is gathered. The correlation measurement used in this method is described in Section 5.3. Section 5.4 contains a description of how the experiments are performed and the method for evaluation is discussed in Section 5.5.

## 5.1 Course of action

The different steps that constitute this method were briefly mentioned in the Aims and objectives, Section 4.3. A more thorough description of the individual steps is discussed below. The development and testing of the hypothesis of this thesis is visually described in Figure 7.

### 5.1.1 Obtaining suitable data

Several different data sets are required, a set of gene expression data as well as a protein chip data set are necessary for the creation of a protein network, Figure 7a). Another data set made up of a protein network is required for the verification of the derived network. Important aspects to take under consideration when collecting data sets are that they are reliable and large enough to create a protein network from. There is a risk that not all proteins present on the protein chip are available in the gene expression data, as well as a low correlation between the genes, which can reduce the size of the protein network.

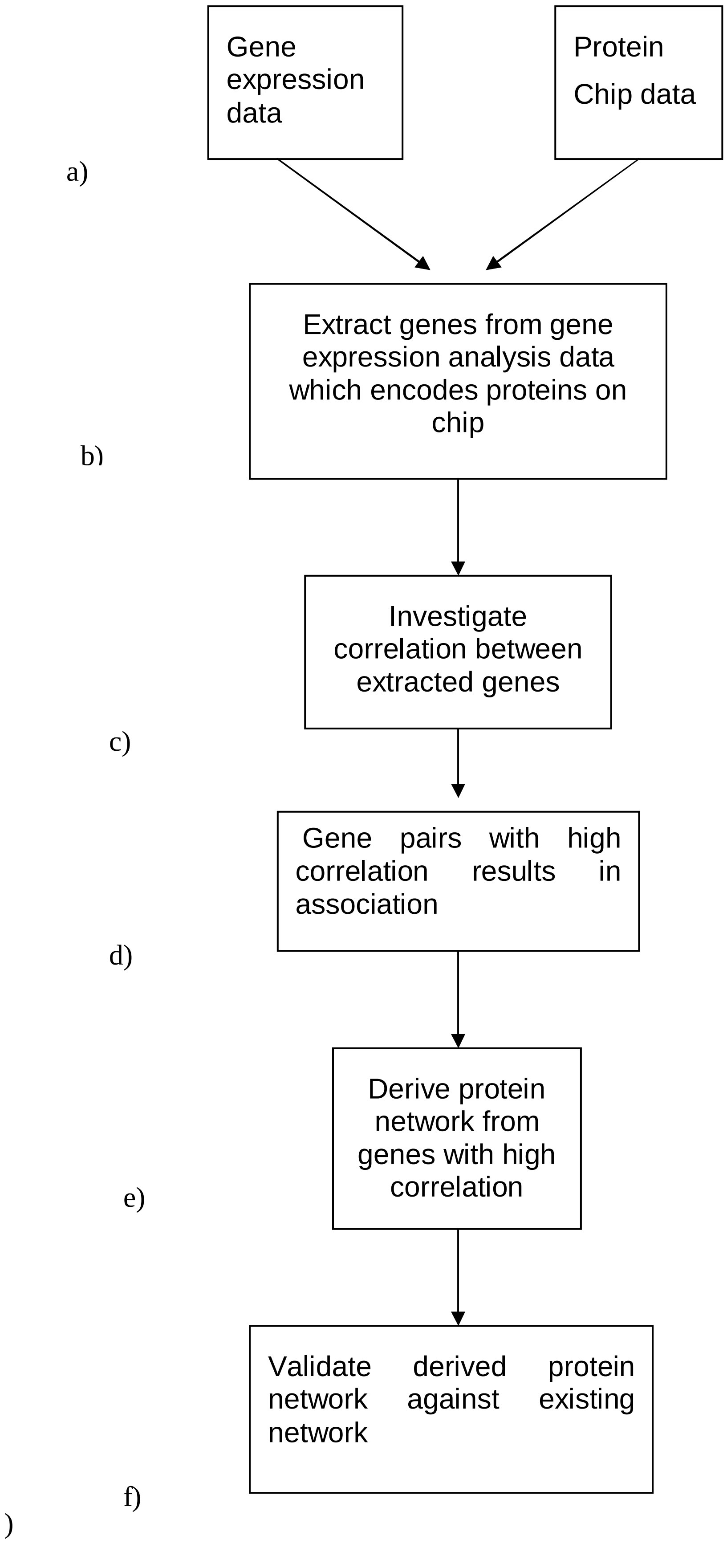

Gene expression data
Protein Chip data
a)
Extract genes from gene expression analysis data which encodes proteins on chip
b)
Investigate correlation between extracted genes
c)
Gene pairs with high correlation results in association
d)
Derive protein network from genes with high correlation
e)
Validate derived protein network against existing network
f)
)

**Figure 7.** An illustration of the course of action.

### 5.1.2 Extraction of genes from gene expression data encoding proteins on chip

The chosen data sets containing protein interaction data and gene expression data respectively are compared in order to extract the genes encoding proteins participating on the protein chip, Figure 7b). It is expected that the majority of the proteins found on the chip have their counterpart genes found in the chosen gene expression data set. The genes that do not encode proteins found on the chip data are then disregarded and not used in forthcoming examinations.

### 5.1.3 Investigate the correlation between genes whose proteins interact on a protein chip

The genes remaining after extraction is used for calculating similarity in expression profiles, Figure 7c). The similarity is measured by using the Pearson correlation coefficient, Section 5.4. The correlation is measured between every pair of gene expression profiles in the remaining data set. By deciding on a specific cut-off, profiles with a correlation similar or higher than this cut-off are kept for further studies. Expression profiles with correlation below chosen cut-off are in this step disregarded. The correlation measurement is used because a high correlation should indicate an interaction, since proteins in the same cellular processes should appear to have similar expression profiles. The Pearson correlation coefficient estimates the similarity in expression profiles. The hypothesis stated in this thesis assumes a high correlation between genes.

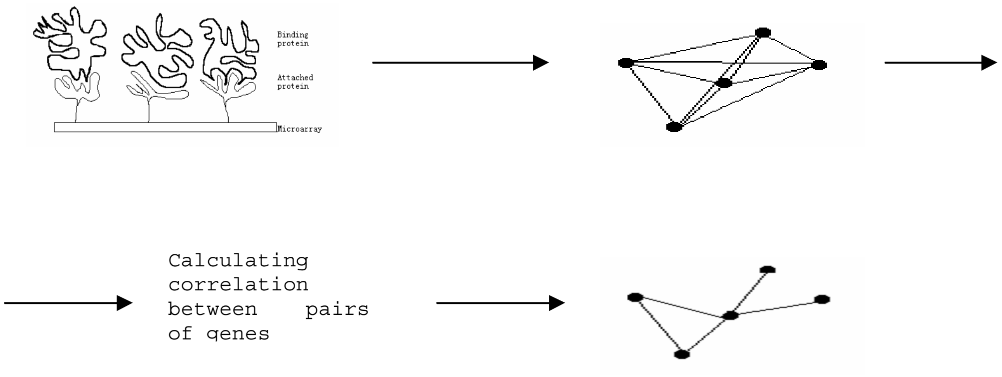


**Figure 8.** Derivation of protein network. The proteins attached to a chip can be used for deriving a protein network. Since it then is impossible to know which proteins are associated with each other, it will be a fully connected network. By using the correlation between genes encoding the proteins the associations will be depending on the correlation. Hence, the protein network will be a less connected protein network.

### 5.1.4 Derivation of protein network

The gene expressions now decide which proteins that are going to participate in the protein network. The proteins utilized are the ones encoded by genes that have a high correlation, Figure 7d). What is now remaining from the original gene expression data set are the genes that both encodes proteins interacting on the protein chip and have a high correlation to other genes encoding proteins on chosen chip. Proteins encoded by these genes are chosen to be part of the derived protein network, Figure 7e). Figure 8 is summarizing the derivation of a protein network.

### 5.1.5 Evaluation of derived protein network

The protein network constructed has to be evaluated in order to receive a satisfying result, i.e. investigate if the derived protein network is reliable, Figure 7f). This evaluation will be done by comparing the derived protein network against an existing network. The comparison consists of two measurements, sensitivity and specificity

(Ideker *et al.,* 2000), Section 5.4. Preferably, both these measurements result in high percentage levels.

## 5.2 Experimental data

The data used for the investigations made in this thesis is collected from publicly available data on the Internet. Data chosen is from the organism *Saccharomyces cerevisiae,* or baker's yeast. This choice is motivated by the fact that the yeast *Saccharomyces cerevisiae* is clearly the most ideal eukaryotic micro-organism for biological studies (Sherman, 1998). A highly versatile DNA transformation system, rapid growth, dispersed cells, the ease of replica plating and mutant isolation are some of the properties that make yeast particularly suitable for biological studies. The complete sequence of its genome has proven to be extremely useful as a reference against the sequences of human and other higher eukaryotic genes. Furthermore, the ease of genetic manipulation of yeast allows its use for conveniently analyzing and functionally dissecting gene products from other eukaryotes (Sherman, 1998).

All data collected is publicly available on the Internet and the intention has been to choose data that is widely studied and used in several different previous experiments. This indicates that the data is more reliable.

### 5.2.1 Gene expression data

There is a huge amount of publicly available gene expression data. Many of these data sets contain gene expression profiles collected from organisms often used for experimental analysis, for example yeast or mouse cells. The different data sets contain data collected at several time points or during different conditions. The number of time points or conditions varies among the data sets.

Gene expression data gives information about the expression patterns of several thousands of genes simultaneously. However, there is always the possibility that these patterns can be somewhat misleading due to noise in the data. This can result in correlations between pairs of genes that do not correspond to the actual patterns. Another aspect to take into consideration is the correlation measurement. The correlation measurement is not always the most proper method for comparing different genes against each other, since it might fail in extracting the best patterns between different genes. Nevertheless, it is a common method for comparing gene expression profiles and no other method has been stated to create more reliable results.

In this thesis, two different data sets have been used.  Both of these data sets contain genes from the budding yeast *Saccharomyces cerevisiae,* but different conditions are studied in the experiments.

The first data set chosen in this thesis is originally performed by DeRisi *et al.*, (1997). This data set has been widely studied and used in many subsequent studies. It contains gene expressions during the diauxic shift, all in all during seven different time points. The microarray used contained essentially every ORF from the yeast *Saccharomyces cerevisiae.*

The second data set is retrieved from Chu *et al.*, (1998). In this article, diploid cells of budding yeast producing haploid cells through the developmental program of sporulation were examined. As well as in the data set from DeRisi *et al.*, seven different time points are studied and DNA micro arrays containing 97 % of the known or predicted yeast genes is used to assay changes in gene expression during sporulation. Time points for taken samples were based on expression patterns of known early, middle, mid-late and late genes in order to receive the most information possible about the expression patterns of genes involved in sporulation.

### 5.2.2 Protein chip data

The amount of publicly available protein chip data is much smaller than the gene expression data. It depends on the relatively new technique for this kind of information retrieval. In the future this information is probably going to be much more frequently found on the Internet.

The data chosen here is collected from Zhu *et al*, (2000). The data set is composed of 119 different yeast protein kinases and their affinity to 17 different substrates on 17 different protein chips. The affinity of the protein kinases to the substrates is indeed interesting, but not further studied in this thesis. Nevertheless, the substrates can be of some significance. Instead, it is the connections between the kinase proteins that are the focus of interest. When investigating all of the kinases and their cellular roles conclusions could be drawn that the majority of the 119 kinases are related in their functions. For that reason, the proteins that bind should have a connection, i.e. they should participate and be closely situated in the same network. The different kinases and their cellular roles can be found in Appendix 1. Section 3.3 contains further information about the experiment.

### 5.2.3 Protein network

The protein network used for validation originates from the article by Schwikowski *et al.* (2000). This network consists of 2 358 interactions between 1 548 proteins of the yeast *Saccharomyces cerevisiae.* Details about the article can be found in Section 3.1.

Since not all these genes are present in the gene expression data and on the protein chip, the network is reduced to only contain the genes relevant, i.e. the network is diminished to only contain the same genes as the ones present in the derived network. Of the 113 genes present on the gene expression data by DeRisi *et al.* (1997), 62 of them are also present in Schwikowskis *et al.* data set and thus used in the evaluation. The same genes that agree with the DeRisi *et al.* data set also agree with the data set from Chu *et al.* (1998). The 62 genes were only connected through 17 associations, which means that not all of them were connected. Only the genes connected to each other are of some interest and therefore only the 20 genes that were connected through the 17 associations were used in the validation. This reduces the derived protein network as well, since not the entire derived network could be validated against these 20 genes.

## 5.3 Correlation measurement

When trying to derive interesting information from the gene expression analysis data, some kind of measurement is required. In order to extract information of whether genes share similar expression patterns, numerous measures are available. Examples of such measures are correlation, Spearman rank correlation, Euclidean distance and the angle between vectors of observations (Heyer *et al.,* 1999). All of the methods have their advantages and disadvantages, for example the Euclidean distance do not score well if the ORF pairs whose expression patterns are measured have the same shape but different magnitudes (Heyer *et al.*, 1999). The Spearman´s rank correlation coefficient is designed for ordinal variables and hence requires ranked values (Heath, 2000) and neither the angle between vectors of observations does not perform high values only for related gene pairs (Heyer *et al.*, 1999).

A widely-used type of correlation coefficient is Pearson correlation. Pearson's correlation reflects the degree of linear relationship between two variables, i.e. it reflects the degree to which the variables are related. In this case the variables are genes. The Pearson correlation identifies positive as well as negative correlation and ranges from +1 to -1. A correlation of +1 means that there is a perfect positive linear relationship between genes and -1 means that there is a perfect negative linear relationship between the genes. Hence, positive correlation identifies similar expression profiles and negative correlation identifies antagonistic expression profiles. A correlation of 0 means there is no linear relationship between the two variables.

Pearson correlation coefficient is defined as:

$$C(x,y) = \frac{\frac{1}{n}\sum_i \left[(x_i - \overline{m}_x)(y_i - \overline{m}_y)\right]}{D(x)D(y)} \qquad \text{eq (1)}$$

where *n* is the number of time points, $x_i$ and $y_i$ are the gene expression levels of *x* and *y* at time *i*, $\overline{m}_x$ and $\overline{m}_y$ are the average expression levels for *x* and *y*, and *D(x)* and *D(y)* are the standard deviations for x and y, respectively.

Since the Pearson correlation coefficient manages both positive and negative correlation it has been the choice of use over for example the Euclidean distance in this method. The most negative aspect with the Pearson correlation is that only linear relationships can be measured, which could affect the outcome.

## 5.4 Experiments

This section provides an overview of the experiments performed in this work. Four different experiments were performed but they were all conducted in the same way. The only differences between the experiments were the data sets used. The two first experiments used two different gene expression data sets, and in the two remaining experiments these two data sets were combined in different ways.

From the original gene expression data set, the genes encoding proteins bound to the substrates on the protein chip were extracted. These remaining genes were further used for calculations of similarity, i.e. the Pearson correlation coefficient was calculated between all pairs of gene expression profiles. This is done since genes with high correlation in this thesis are thought to share the same regulation and hence be part of the same protein network.

The Pearson correlation coefficient is used for defining the associations between proteins when constructing a network. Every single association between two proteins in the derived protein network exists only where the correlation value between their encoding genes exceeds the chosen correlation cut off value. Since there is no standard in choosing cut off, several different values were tested. Here, three different cut offs were used to distinguish the associated genes: $|0.6|$, $|0.7|$ and $|0.8|$. Using different cut offs results in protein networks differing in appearance and hence it is an important task finding the most appropriate cut off value. If the cut off is set too low, the network will consist of too many false positive associations. Otherwise, if the cut off instead is set too high, many associations that is true will not be found and the protein network will be missing important information.

Proteins encoded by genes that have correlations above chosen cut off are consequently participating in the derived protein network. The cut off chosen discriminates correct associations from falsely predicted associations.

Gene expression profiles depend on the situation when the expressions are measured, different profiles is produced during cell-cycle and diauxic shift as well as during sporulation. The protein chip data is not dependent on any situation, and hence there can exist associations despite a low correlation.

Networks created accordingly have to be validated, and the protein network created by Schwikowski *et al*. (2000) is used for this purpose. The protein network used as verification is considerably larger than the derived network, and hence containing several genes that were irrelevant considering the networks derived using the method and data sets described earlier. Only a fraction of the genes present in the

Schwikowski *et al.* network is also present in the derived networks. Therefore, in order to make the Schwikowski *et al.* (2000) network more relevant and efficient considering analysing the results, genes not participating in the derived networks were to be excluded. In this manner, analysis of the results was made easier, since no respect had to be taken to irrelevant genes. Hence, the data set collected from Schwikowski *et al*. (2000) originally contained 1 548 proteins, but was reduced to only consider the genes participating in the derived protein networks.

The associations between the proteins participating in the known protein network were compared against the associations constituting the networks derived by the method developed in this thesis. Not all the proteins that together creates the derived protein network is evident to be found in the protein network developed by Schwikowski *et al.* (2000). Although the verified network consists of many genes there is a possibility that all the genes participating in the derived networks not exists in the verified network anyhow. Hence, the derived network possibly has to be reduced to only contain the genes also present in the validation network by Schwikowski *et al.* (2000) in order to make the validation possible for all these putative interactions.

The method for comparison is the sensitivity and specificity measurement, Section 5.4. The number of existing associations relative the number of identical associations in the derived and verified networks is studied in order to find the ratio between them. This gives an indication whether the derived method for constructing protein networks is reliable.

## 5.5 Method for evaluation

The performance of the method is evaluated by comparing the derived network against an already known, reliable network. The comparison between the two networks consists of a method developed by Ideker *et al.* (2000). Two measurements were developed in order to specify the correctness of the developed network (Figure 9). The measurements were called sensitivity and specificity, respectively, and are defined as:

**sensitivity:** the percentage of edges in validated network that are also present in the derived network

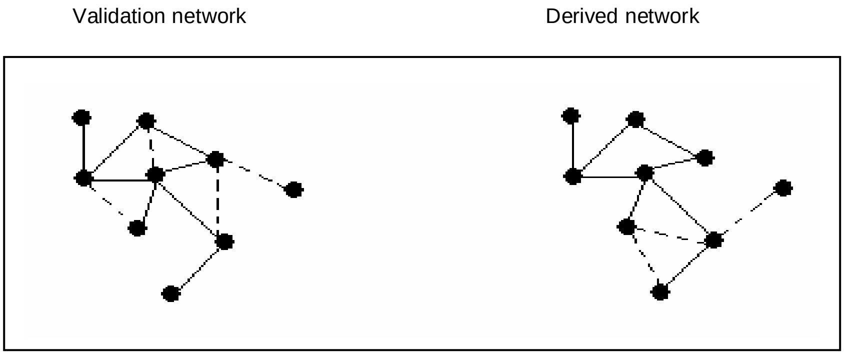
**specificity:** the percentage of edges in the derived network that are also present in the validated network

The validated network in this case is the protein network that is used for validation of the derived protein network. It is desirable for both the sensitivity and specificity to have high percentage levels, since it indicates that the derived network is highly similar to the validation network. This measurement is chosen because of its ease of use and the lack of complex calculations required.

The sensitivity and specificity measurements are dependent on the size of the networks compared. This affects the results when calculating the sensitivity and specificity. If the protein networks are small and, it is easier to find the right connections and hence the sensitivity and specificity will increase. If instead the protein networks are large it is possible that the measurement values decreases.

$$\text{Sensitivity} = \frac{\text{Number of edges in validated network present in derived network}}{\text{Total number of edges in validated network}} = 8 / 11 = 0.73$$

$$\text{Specificity} = \frac{\text{No of edges in derived network present in validated network}}{\text{Total number of edges in derived network}} = 8 / 10 = 0.80$$



| | |
|---|---|
| Total number of connections in validation network: | 11 |
| Total number of connections in derived network: | 10 |
| Number of identical connections: | 8 |

**Figure 9.** Ideker *et al.* (2000) developed the sensitivity and specificity measurement. The solid lines are edges existing in both networks, while dashed lines are edges specific to respective network.

# 6 Results and analysis

When examining the hypothesis stated in this thesis, three different experiments were performed. The experiments were executed in the same way and all of the experiments used the same protein chip data originally collected from Zhu *et al.* (2000). The significant difference between the tests was that two different gene expression data sets were used. The data sets were first examined individually and in the third experiment they were combined in two different ways. By testing the hypothesis more than once, the probability of the results increases strongly. In Section 6.1 the experiment performed with gene expression data from DeRisi *et al.* (1997) is thoroughly described along with an analysis of the results. This experiment is followed by the experiment using data from Chu *et al.* (1998) in Section 6.2. Also here an analysis of the results is enclosed. Section 6.3 considers experiments combining the two gene expression data sets used in the previous experiments.

All the results are summarized in Appendix 5.

## 6.1 Experiment 1

In this experiment the DeRisi *et al.* (1997) data set is made use of together with the protein chip data set from Zhu *et al.* (2000).  DeRisi *et al.* created a gene expression data set containing almost the entire set of genes from yeast. A reduction from these approximately 6 400 genes down to 113 genes was performed in order to make the data set contain only the genes corresponding to the proteins on the protein chip. Appendix 2 contains the genes selected. The protein chip data contains 119 proteins and hence the corresponding gene for six of the proteins was not to be found.

Thereafter, the Pearson correlation was calculated between the 113 genes for the purpose of similarity studies. In order to investigate if the correlation between genes was strong, different cut offs for the correlations were examined. The various cut offs gave different results (Table 1); when using a cut off of │0.6│, all the 113 genes extracted from the gene expression data participated in one single large network and generated 1 296 associations. This means that all genes in some way, through different amount of interactions, are to be traced to each other. On average the genes are directly connected to 11.5 other genes.

When using the cut off │0.7│ all genes except four were associated within 564 associations. The proteins associated within these 564 connections were participating in a single protein network. The four proteins not participating in these associations were not connected to this network; they were only connected to each other in two separate pairs. If studying the number of connections between all the 113 genes, the number of connections accordingly increased to 566. This means that each gene instead, on average, is connected directly to 5.0 other genes which is a significantly more loosely connected network. By increasing the cut off to │0.8│, a drastic difference was observed. No single large network was to be noticed. Instead, 11 different networks appeared and the smallest ones only containing a single pair of genes. The largest network consisted of 21 different genes that shared 32 associations.,

Between all genes, the number of associations was counted to 54, i.e. the average number of connections for each gene was 0.5.  When disregarding that the genes did not end up in a single network in two out of the three derived networks, the sum of connections is still prominently decreasing.

| **Cut off** | **│0.6│** | **│0.7│** | **│0.8│** |
|---|---|---|---|
| **Number of derived associations in data set from DeRisi *et al.* (1997)** | **1296** | **564** | **54** |
| **Number of *reduced* associations in data set from DeRisi *et al.* (1997)** | **36** | **17** | **3** |

**Table 1.** The different cut offs results in networks with a different amount of associations. Increasing the value of cut off gives protein networks significantly more loosely connected. Also the number of associations possible to validate against the data set from Schwikowski *et al.* (2000) is shown in row three.

When using the gene expression data from DeRisi *et al.*, 113 of the 119 proteins had their encoding gene in this data set. Of these 113 genes, 62 are present in the verified protein network by Schwikowski *et al.* (2000), (Appendix 3). Hence, only these 62 proteins in the gene expression data are studied, with the possibility to be evaluated against the Schwikowski *et al.* network. In the verified network, 17 associations are detected between these 62 genes. Since it is not possible for 62 different proteins to be connected with only 17 different associations, the conclusion can be drawn that all of these 62 genes are not connected. The only genes interesting for further studies are those genes connected by the associations, since genes that do not form connections with any other genes neither participate in a network, (Appendix 4).

The number of genes connected with the 17 associations was only counted to 20. These twenty genes are the only ones that can be validated using the protein network by Schwikowski *et al.* (2000) as evaluation method. When calculating sensitivity and specificity for the derived network, only these twenty genes are considered. The number of associations found in the derived network, considering this diminished set of genes was 36, 17 and 3 respectively for the three different cut offs used.

To sum up, the validation network was in several steps reduced from 1 548 genes and 2 358 associations down to 20 genes and 17 associations. On the same time as the gene expression data set was reduced from ~ 6 400 to the same 20 genes. Consequently, the protein chip data set that originally contained 119 protein kinases was reduced to the 20 proteins encoded by the 20 genes possible to validate.

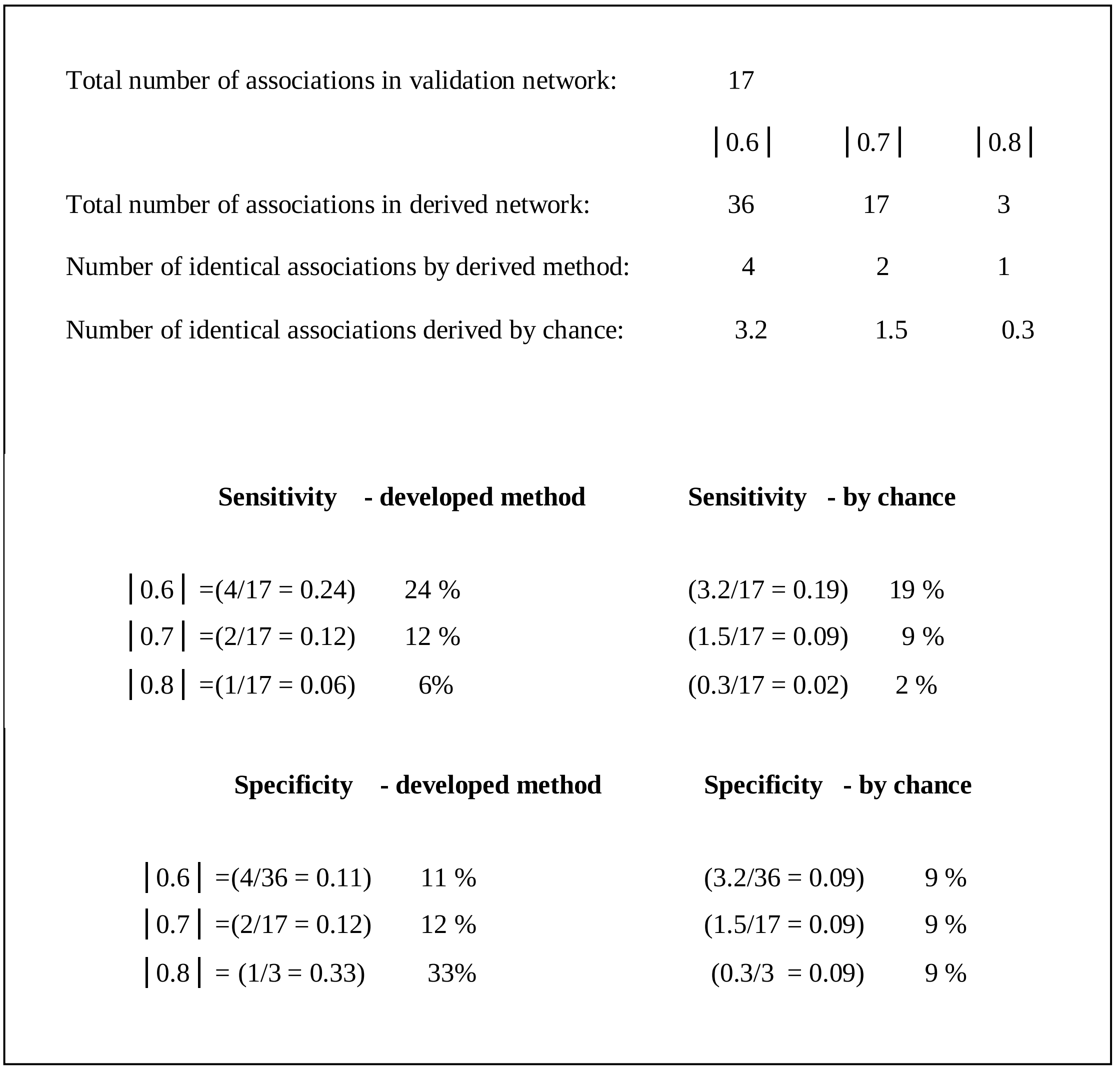
In order to be able to calculate the sensitivity and specificity, the number of associations identical among the remaining 20 genes in the both networks had to be counted, (Table 2). An identical, or verified, association is an association between two genes, and it exists in both networks. The number of verified associations when the cut off is determined to $\lvert 0.6 \rvert$ are four and is decreased to two when the cut off is $\lvert 0.7 \rvert$, (Appendix 4). Only a single association is verified when the cut off thereafter is changed to $\lvert 0.8 \rvert$.

| **Cut off** | **$\lvert 0.6 \rvert$** | **$\lvert 0.7 \rvert$** | **$\lvert 0.8 \rvert$** |
|---|---|---|---|
| **Number of verified genes in derived and validated protein network** | **4** | **2** | **1** |

**Table 2**. Showing the number of associations that are found in both the data set from DeRisi *et al.* (1997) and in the validation data from Schwikowski *et al.* (2000).

This means that the sensitivity and specificity respectively is 0.24 and 0.11 when cut off is set to $\lvert 0.6 \rvert$, see Figure 10 for calculations. Having a sensitivity of 24 % means that almost every fourth association in the data from Schwikowski *et al.* (2000), is also present in the derived network. The specificity of 11 % declares that only eleven of a hundred associations in the derived network are also present in the validation network. The specificity is almost constant, specificity equals 12 %, when the cut off is changed to $|0.7|$, but the sensitivity is reduced to half, i.e. 12 %. When increasing the cut off to $|0.8|$, the sensitivity is reduced by half once again, down to 6 % while the specificity is improved to 33 % (Figure 10).

The sensitivity and specificity measurement is somewhat difficult to interpret and in order to understand what these figures say, they should be compared to what can be expected by chance. 20 genes were used and a fully connected network of that size would generate 20*19/2 (190) associations. In the protein network by Schwikowski *et al.* (2000), only 17 associations were found. That is 9 % of the fully connected network. In the derived network, 36, 17 respectively 3 associations were found. When generating 36 associations by chance among the 190 possible associations, the result should be about 3.2 correct associations (Figure 10 ). The resulting sensitivity and specificity should hence be 19 % respectively 9 % when deriving associations by chance. Comparing these results (19 % and 9 %) with the results generated with the developed method (24 % and 11 %), there is no evident difference. When doing these calculations for the cut offs $|0.7|$ and $|0.8|$ as well, the sensitivity is by chance 9 % respectively 2 %. All these results indicate that the method developed in this thesis is only marginally better in performance than deriving protein networks by chance.



| | | | |
|---|---|---|---|
| Total number of associations in validation network: | 17 | | |
| | │0.6│ | │0.7│ | │0.8│ |
| Total number of associations in derived network: | 36 | 17 | 3 |
| Number of identical associations by derived method: | 4 | 2 | 1 |
| Number of identical associations derived by chance: | 3.2 | 1.5 | 0.3 |

| | **Sensitivity - developed method** | | **Sensitivity - by chance** | |
|---|---|---|---|---|
| │0.6│ | =(4/17 = 0.24) | 24 % | (3.2/17 = 0.19) | 19 % |
| │0.7│ | =(2/17 = 0.12) | 12 % | (1.5/17 = 0.09) | 9 % |
| │0.8│ | =(1/17 = 0.06) | 6% | (0.3/17 = 0.02) | 2 % |

| | **Specificity - developed method** | | **Specificity - by chance** | |
|---|---|---|---|---|
| │0.6│ | =(4/36 = 0.11) | 11 % | (3.2/36 = 0.09) | 9 % |
| │0.7│ | =(2/17 = 0.12) | 12 % | (1.5/17 = 0.09) | 9 % |
| │0.8│ | = (1/3 = 0.33) | 33% | (0.3/3 = 0.09) | 9 % |

**Figure 10.** A description of the calculations performed in order to compare the developed method against the performance when deriving protein networks by chance.

## 6.2 Experiment 2

This second experiment was carried out in the same manners as the first experiment. The same protein chip data by Zhu *et al.* (2000) were used, but the gene expression data that together with the protein chip data constituted the foundation when constructing the protein networks, was a different set. This gene expression data set is originated from the article by Chu *et al.* (1998) and is composed of genes from *Saccharomyces cerevisiae* studied during sporulation. The data set constructed by

Chu *et al*, originally contained approximately 6100 genes and these had to be reduced to a set only containing genes found on the protein chip. Out of the 119 protein kinases found on the chip, 109 of them were also found in the gene expression data from Chu *et al.* (1998). Already at this stage, the proteins constituting the final network was diminished by ten proteins.

Next step was to calculate the Pearson correlation between the remaining 109 genes. The same cut off values were used as in the first experiment, i.e. $|0.6|$, $|0.7|$ and $|0.8|$. As in the first experiment, the number of associations decreased with the increase of cut off value. When having the cut off set to $|0.6|$, the number of associations was 1425, which means that each gene on average is connected to 13.1 other genes. When increasing the cut off to $|0.7|$, the associations dropped to 696, meaning an average of 6.4 associations for each gene. The average number of associations decreased further down to 0.7 when cut off value equals $|0.8|$, since totally only 83 associations were found. D'haeseleer *et al.* (2000) stated that each gene or protein is estimated on average to interact with four to eight other genes or proteins. When having a cut off value of $|0.6|$, the average number in this experiment is 13.1 other genes. In the first experiment the number of average connected genes was 11.5. This indicates that the cut off $|0.6|$ not is a good choice of cut off value. Instead it would be increased. The number of connections when having the cut off $|0.7|$ is more accurate considering the number of genes connected to each other.

In all three cases the derived protein networks were more strongly connected, i.e. more associations existed amongst fewer proteins than in the first experiment. A probable consequence would be that the number of networks produced, especially when using the cut off value │0.8│, should be fewer. When examining this, the conclusion was right, although with a small marginal, since ten different networks was created instead of eleven as in the experiment using data from DeRisi *et al.* (1997).

In order to validate the protein networks constructed in this experiment, the protein network performed by Schwikowski *et al.* (2000) was used for validation, exactly like in the previous experiment. When designating which genes could be found in both the constructed network and the network by Schwikowski *et al.* (2000), exactly the same 62 genes as in the first experiment was found in the validation network. These genes are found in Appendix 3.

Obviously, the same 17 associations amongst the 62 genes from Schwikowski *et al,* (2000) were still present and the genes associated by these 17 associations were selected from this collection, (Appendix 4). The same 20 genes as in the first experiment was connected by these 17 associations and appointed for forthcoming studies. The derived protein network was then reduced to contain only the genes possible to evaluate as well. As a consequence, the number of associations in the derived network decreased remarkably, (Table 3).

| **Cut off** | **│0.6│** | **│0.7│** | **│0.8│** |
|---|---|---|---|
| **Number of derived associations in data set from Chu *et al.* (1998)** | **1425** | **696** | **83** |
| **Number of *reduced* associations in data set from Chu *et al.* (1998)** | **29** | **8** | **2** |

**Table 3.** Different cut off values creates different protein networks. The number of associations is illustrated together with the number of associations possible to verify.

Studying the associations in order to find how many of them that could be found in the derived protein network as well as in the validation network, five verified associations were encountered with the cut off |0.6|, (Table 4). Increasing the cut off resulted in that the number of verified associations decreased to 1 for both of the remaining cut offs. The five different pairs of genes found in both data sets were not the same in the both experiments. None of the four identical associations found in the first experiment were found in this experiment. On the other hand, five completely new pairs of genes were detected instead.

When calculating sensitivity and specificity, the results appeared to be quite similar to the results given in the first experiment, (Figure 11). The specificity when using cut off |0.6|, gave approximately 17 %, and when using |0.7| as cut off, the specificity is decreased to 12 %. When changing cut off to |0.8|, the specificity is dramatically improved and ends up on 50 %, which depends on that it is one association out of two possible. This number of associations is too small for drawing any reliable conclusions. These results were somewhat slightly better than in the first experiment, on the contrary to the sensitivity measures. Cut off |0.6| showed a sensitivity of 29 %, which is the only sensitivity result better in this experiment than in the first one. |0.7| and |0.8| as cut off values gave both sensitivities of 6 %, which is half of the resulting value on the sensitivity measured in the first experiment using cut off set to |0.7|.

| **Cut off** | **│0.6│** | **│0.7│** | **│0.8│** |
|---|---|---|---|
| **Number of verified associations in derived and validated protein network** | **5** | **1** | **1** |

**Table 4**. Showing the number of associations that are found in both the data set from Chu *et al.* (1998) and in the validation data from Schwikowski *et al.* (2000).

Also here the sensitivity and specificity measurements were compared when calculations were made from results derived from the developed method and results derived by chance, (Figure 11). The values of sensitivity derived by chance for the different cut offs are in order: 15 %, 4 % and 1%. There is a more evident difference between these figures than there were between the sensitivity measures in experiment 1, which indicates that this data set is performing somewhat better.

Total number of associations in validation network: 17

| | │0.6│ | │0.7│ | │0.8│ |
|---|---|---|---|
| Total number of associations in derived network: | 29 | 8 | 2 |
| Number of identical associations by derived method: | 5 | 1 | 1 |
| Number of identical associations derived by chance: | 2.6 | 0.7 | 0.2 |

| | **Sensitivity - developed method** | | **Sensitivity - by chance** | |
|---|---|---|---|---|
| │0.6│ | =(5/17 = 0.29) | 29 % | (2.6/17 = 0.15) | 15 % |
| │0.7│ | =(1/17 = 0.06) | 6 % | (0.7/17 = 0.04) | 4 % |
| │0.8│ | =(1/17 = 0.06) | 6 % | (0.2/17 = 0.01) | 1 % |

| | **Specificity - developed method** | | **Specificity - by chance** | |
|---|---|---|---|---|
| │0.6│ | =(5/29 = 0.17) | 17 % | (2.6/29 = 0.09) | 9 % |
| │0.7│ | = (1/8 = 0.12) | 12 % | (0.7/8 = 0.09) | 9 % |
| │0.8│ | = (1/2 = 0.50) | 50 % | (0.2/2 = 0.09) | 9 % |

**Figure 11.** A description of the calculations performed in order to compare the developed method against the performance when deriving protein networks by chance.

## 6.3 Experiment 3

The third experiment is divided into two different experiments, both of them combining the data sets used in the first and second experiments in different ways.

### 6.3.1 Experiment 3a

In order to improve the sensitivity and specificity, i.e. improve the performance, the two gene expression data sets were combined. Since it is clear from the first and second experiments which twenty genes that are possible to validate, only these genes were investigated. The expression profiles from the both data sets for the chosen genes were concatenated into one data set, which was used in the same way as they were used separately in the previous two experiments. The Pearson correlation was calculated and the result is slightly better when combining the two sets compared to investigate them one by one (Figure 12).

The number of associations found using the cut offs │0.6│, │0.7│ and │0.8│ are 31, 23 and 4 respectively, (Table 5). The associations found verified in the combined data set and the validation data set were 5, 4 and 1, respectively for the three different cut offs, (Table 5). Three of these verified associations were identical also in the first experiment, when only the DeRisi *et al.* (1997) data set was used. One association were identical in this experiment as well as in the second experiment performed with the data set originated from Chu *et al.* (1998), and one identical association were not to be found in either of the data sets on their own. One might think that the verified associations to be found would be the nine different associations found identical when examining the gene expression data sets by their own in the first and second experiments. This is evidently not the case and it might depend on the expression profiles. It can also depend on noise in the data sets or that the different data sets expresses profiles from different situations. The data set from DeRisi et al. (1997) considers the yeast genes during diauxic shift whereas the Chu et al. (1998) data set studies the same genes during sporulation. Probably, the profiles from the different sets are very dissimilar. So, even if the expression profiles from one of the data sets is very similar for two genes and results in a very high correlation for that experiment, the profiles extracted from the other data set can differ from each other very strongly. When these expression profiles then are combined, it causes the Pearson correlation to decrease to the extent that it ends up being lower than chosen cut off. The associations is then no longer part of the protein network.

| **Cut off** | **│0.6│** | **│0.7│** | **│0.8│** |
|---|---|---|---|
| **Number of verified associations in the combined data set from Chu *et al.* (1998) and DeRisi *et al.* (1997)** | **31** | **23** | **4** |
| **Number of identical associations in the combined data set and the data set from Schwikowski *et al.* (2000)** | **5** | **4** | **1** |

**Table 5.** The number of associations found among the twenty selected genes and the associations among these found identical with associations in the data set from the validation set from Schwikowski *et al.* (2000).

Results when calculating the sensitivity and specificity are shown in Figure 12. The sensitivity is somewhat better in this third experiment than in the previous two experiments. The sensitivity is performing about twice as good as if the associations were derived by chance, but still a sensitivity of 29 % as the best result is not satisfying.

The specificity is also just marginally better when combining the data sets, and compared to specificity by chance it is performing almost twice as good. Something that can be worth mentioning is that the specificity for cut off │0.8│ has decreased to 25 %, but since there are more associations involved this figure is more reliable.

Total number of associations in validation network: 17

| | \|0.6\| | \|0.7\| | \|0.8\| |
|---|---|---|---|
| Total number of associations in derived network: | 31 | 23 | 4 |
| Number of identical associations by derived method: | 5 | 4 | 1 |
| Number of identical associations derived by chance: | 2.8 | 2.1 | 0.4 |

| | **Sensitivity - developed method** | | **Sensitivity - by chance** | |
|---|---|---|---|---|
| \|0.6\| | =(5/17 = 0.29) | 29 % | (2.8/17 = 0.16) | 16 % |
| \|0.7\| | =(4/17 = 0.24) | 24 % | (2.1/17 = 0.12) | 12 % |
| \|0.8\| | =(1/17 = 0.06) | 6 % | (0.4/17 = 0.02) | 2 % |

| | **Specificity - developed method** | | **Specificity - by chance** | |
|---|---|---|---|---|
| \|0.6\| | =(5/31 = 0.16) | 16 % | (2.8/31 = 0.09) | 9 % |
| \|0.7\| | =(4/23 = 0.17) | 17 % | (2.1/23 = 0.09) | 9 % |
| \|0.8\| | = (1/4 = 0.25) | 25 % | (0.4/4 = 0.09) | 9 % |

**Figure 12.** The sensitivity and specificity for the derived networks when the two data sets are merged.

### 6.3.2 Experiment 3b

Here, instead of calculating a new correlation value for the affected genes using both the expression data sets, another way of analysing a combination of the data sets is performed. The two data sets are combined through the results from the first and second experiment together. In the first experiment (Table 6), five identical associations were found while four different associations were found in the second experiment when using cut off |0.6|. If adding these associations together, there would be nine different associations identical to the ones found in the validation data from Schwikowski *et al.* (2000).

| **Cut off** | **│0.6│** | **│0.7│** | **│0.8│** |
|---|---|---|---|
| **Number of identical associations when the two data sets are added together.** | **9** | **3** | **2** |

**Table 6.** The number of identical associations in the two different data sets was added together. No identical association were found in data sets from both DeRisi *et al.* (1997) and Chu *et al.* (1998).

In order to calculate the sensitivity and specificity, the number of associations in the derived networks has to be counted and added together. There would then be 65 (29 + 36) associations, but since three of these associations occurred in both data sets the final number is 62, for cut off │0.6│. Cut off │0.7│and│0.8│gave 24 respectively 5 associations. When combining the data sets in this manner, a different appearance occur for the sensitivity and specificity, (Figure 13). The results from this experiment gave the best figures of all the experiments done in this thesis, except for the specificity for │0.6│.

By combining the two different data sets in different ways, the results differ. This indicates that different ways of combinations is relevant to do. It is not obvious that one way of combining is always better than the other and hence it is not always the best thing to concatenate different data sets into one, which is commonly done.

Total number of associations in validated network: 17

| | │0.6│ | │0.7│ | │0.8│ |
|---|---|---|---|
| Total number of associations in derived network: | 62 | 24 | 5 |
| Number of identical associations by derived method: | 9 | 5 | 2 |
| Number of identical associations derived by chance: | 5.6 | 2.2 | 0.5 |

| | **Sensitivity - developed method** | | **Sensitivity - by chance** | |
|---|---|---|---|---|
| │0.6│ | =(9/17 = 0.53) | 53 % | (5.6/17 = 0.33) | 33 % |
| │0.7│ | =(5/17 = 0.29) | 29 % | (2.2/17 = 0.13) | 13 % |
| │0.8│ | =(2/17 = 0.12) | 12 % | (0.5/17 = 0.03) | 3 % |

| | **Specificity - developed method** | | **Specificity - by chance** | |
|---|---|---|---|---|
| │0.6│ | =(9/62 = 0.15) | 15 % | (5.6/62 = 0.09) | 9 % |
| │0.7│ | =(5/24 = 0.21) | 21 % | (2.2/24 = 0.09) | 9 % |
| │0.8│ | = (2/5 = 0.40) | 40 % | (0.5/5 = 0.09) | 9 % |

**Figure 13.** Sensitivity and specificity calculated when the numbers from the two data sets are added together.

# 7 Discussion

The purpose of this work was to derive a method for constructing reliable protein networks by combining protein chips with gene expression analysis. The intention of combining different approaches is to create protein networks using more than one source and hence obtain networks that are more accurate than those constructed from only a single source. The effect of combining different methods were hoped to diminish results that are not correct, depending on mistakes or deficiencies in the method used. Considering these circumstances, the method derived in this thesis was expected to perform better than has been proven by the experiments performed.

The sensitivity and specificity measurements that were used for the validation gave poor results for the method derived in this thesis. Factors that can affect the results are that the data sets were too small or that the network used for validation not contained enough genes, which can cause the validation to be performed on very few genes. If the validation is done on only a small fraction of genes it can be far from reliable, since the risk that the proportions can be misleading increases.

The major drawback in this thesis is that the different data sets are not matching, and that applies mainly to the validation set from Schwikowski *et al.* (2000). Out of the 1 548 proteins participating in this network, only 62 of them were found at all in the derived network, and this figure was diminished down to 20 that actually were possible to validate against. This is too few proteins when trying to falsify the hypothesis. The different data sets should have been more thoroughly studied before the experiments were performed to see if they were compatible against each other. If that had been done,  data sets could have been used that contained more of the same genes and proteins.

It was not only the dataset from Schwikowski *et al.* (2000) that made the experiment results so poor. In the first place, the protein chip data from Zhu *et al.* (2000), which the whole experiment started from, only contained 119 proteins. This is a very restricted amount of data to start with. Another drawback that was not counted for was the extent of reduction of these proteins that actually took place. A protein network consisting of all the 119 proteins the experiments started with is perhaps not a large network, but if all these proteins could be found in and validated against the other data sets, the results would be much more reliable.

In addition, it is not only the validation data set that has caused problems. There are also aspects needed to be respected considering the other data sets. Not all the genes encoding the proteins found on the chip were found on the gene expression data sets that were used. This as well reduces the number of proteins that possibly should participate in the derived network.

Another aspect that can explain why the last two experiments where the two gene expression data sets were combined performed so poorly, can be that the different data sets considers different situations. Since genes are expressed differently during diauxic shift and sporulation the concatenation of these expression profiles can be misleading.

The sensitivity and specificity is very low, but there are several reasons for that, which affect the reliability of the hypothesis. For example, it is assumed that the Schwikowski *et al.* (2000) network is true and reliable, but it does not have to be complete. Not all the connections that exist between the proteins that together construct this network are known. A great amount of information about these connections is still missing or is not public information. If there were more knowledge about associations between proteins this perhaps could increase the sensitivity and specificity for the method derived in this thesis.

In order to increase the values of the sensitivity and specificity measures, literature studies should be performed. In this way there is a possibility to come across many associations not found in the validation network. Hence, the number of associations and proteins connected by these associations could be increased and used for validating parts of the derived networks that were not able to be validated using the Schwikowski *et al.* network. The hypothesis would be more reliable if there was a possibility of increasing the sensitivity and specificity. As it looks right now, the method does not perform much better than if the networks were derived by chance.

If proteins chosen to be included in the network because of their similarity in expression patterns should appear not to be adjacent in the derived protein network, the distance between them could be calculated. The assumption is being made that proteins encoded by genes with similar expression pattern then is at least located near each other in the network. This could give indications on the appearance of the protein networks.

The gene expression profiles are measured during different time points and conditions. Hence, the resulting data are dependent on those time points and conditions. The data from the protein chips are not dependent of these aspects and this can cause the results to be less accurate. Since the correlation between different genes differ during different conditions it is important to take into consideration which conditions that are studied. The results might have been better if the gene expression data came from time points during the cell cycle.

Protein kinases are phosphorylating proteins and are often responsible for post-translational modifications of other proteins. This is not always expressed in genetic or protein networks. Since the protein kinases not always are commonly expressed in these networks, it might be a higher degree of agreement between the derived results and a true metabolic network than has been revealed in this thesis.

## 7.1 Future work

When comparing the first two experiments with the ones where the two gene expression data sets were combined, the conclusion is that the combined data sets are performing slightly better. This indicates that as much information as possible should be used, since the chances of getting better results then increases. The most information is retrieved by using several different types of experiments, instead of huge amounts of data from similar experiments. These different types reflect different reactions, and can consequently complement each other in order to achieve large amounts of valuable data.

So, a consequence of this would be to investigate the hypothesis stated in this thesis with a larger protein data set as well as a larger gene expression data set. The chances of constructing a network with more genes that is possible to evaluate, then increases.

A procedure that could increase the amount of proteins possible to validate in the derived protein network would be to supplement the Schwikowski *et al.* validation network with literature surveys. This could improve the results since the results now only are based on twenty proteins, which actually is far too few in order to derivate reliable results on. The literature surveys can make it possible to find associations not found in the validation network.

It would also be of interest to validate the derived networks against another protein network containing more genes that can be found in the data sets used when deriving the protein networks.

Another interesting aspect to continue with would be to see how much the gene expression data actually contribute to increase the reliability of the derived protein network. Assuming that all the proteins found on the protein chip can be put together in a single network, is it then possible to verify all these associations with the help of the protein network by Schwikowski *et al.* (2000)? How much do the gene expression data affect the result, is it getting better or even worse?

Since so few of the associations derived were to be found in the validation network, it would be of interest studying the distance between the different genes that had a high correlation. It could be of significant interest if the genes with high correlation instead of being directly connected, at least were situated near each other in the network. This would indicate the method derived possibly could give some hints about the appearance of the network.

# 8 Conclusions

The hypothesis stated in this thesis is that the combination of protein chips and gene expression data can predict reliable protein networks. This hypothesis could not, with the experiments and the validation performed here, be said to be true. But, since the experiments performed not are rigorous enough, it would be a rash conclusion if the hypothesis was altogether falsified. The hypothesis could not be considered completely worthless since the method derived some true associations, but too many false positive associations were derived as well. Consequently, the hypothesis is still unanswered, but the method validated in this thesis is at the moment not to be stated successful. There are many factors affecting the results, and that need to be investigated further. The main factors that have affected the results above all, are the mismatching and shortage of data. Therefore, it is necessary to see how the method performs with data more suitable for examining this method. Further investigations are required.

The approach developed for deriving protein networks is intriguing, but there are some aspects that can affect the results negatively. One of the major obstacles is that the protein chip data is not depending on different time points or conditions, as the gene expression data are. Maybe there are ambiguities when combining the different data sets, or the information gained is not good enough since they are not depending on the same conditions.

# References

Abbott, A. (2002) Betting on tomorrow's chips. *Nature, 415,* 112-114.

Brazma, A. and Vilo, J. (2000) Gene expression data analysis. *Federation of European Biochemical Societies, 480,* 17-24.

Campbell, N. A., Reece, J. B. and Mitchell L. G. (1999) *Biology* (5th Edition). Addison Wesley Longman, Inc. USA.

Chu, S., DeRisi, J., Mulholland, J., Botstein. D., Brown, P. O. and Herskowitz, I. (1998) The Transcriptional Program of Sporulation in Budding Yeast. *Science, 282,* 699-705.

DeRisi, J. L., Iyer, V. R. and Brown, P. O. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science, 278,* 680-686.

D'haeseleer, P., Liang, S., and Somogyi, R., (2000) Genetic Network Inference: From Co-Expression Clustering to Reverse Engineering**.** *Bioinformatics 16(8), 707-726.*

D'haeseleer, P., Wen, X., Fuhrman, S., and Somogyi, R. (1999) Linear Modeling of mRNA expression levels during CNS development and injury**.** *Pacific Symposium on Biocomputing ´99,* 41-52. World Scientific Publishing Co.

Edwards, J. S. and Palsson. B. O. (2000) Robustness Analysis of the *Escherichia coli* Metabolic Network. *Biotechnol Prog., 16,* 927-939.

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

Fannon, M. R. (1996) Gene expression in normal and disease states- identification of therapeutic targets. *Trends in Biotech, 14,* 294-298.

Gavin, A-C., Bösche M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J. M., Michon, A-M., Cruciat, C-M., Remor, M., Höfert, C., Schelder, M., Brajenovic, M., Ruffner, H., Merino, A., Klein, K., Hudak, M., Dickson, D., Rudi, T., Gnau, V., Bauch, A., Bastuck, S., Huhse, B., Leutwein, C., Heurtier, M-A., Copley, R. R., Edelmann, A., Querfurth, E., Rybin, V., Drewes, G., Raida, M., Bouwmeester. T, Boork, P., Seraphin, B., Kuster, B., Neubauer, G and Superti-Furga, G. (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature, 415,* 141-147.

Gustafsson, A. (2002) Proteiner på chip genomskådar sjukdomar. *NyTeknik, 6,* 19.

Ho, Y., Gruhler, A., Heilbut, A., Bader, G. D., Moore, L., Adams, S-L., Millar, A., Taylor, P., Bennett, K., Boutilier, k., Yang, L., Wolting, C., Donaldson, I., Schandorff, S., Shewnarane, J., Vi, M., Taggart, J., Goudreault, M., Muskat, B., Alfarno, C., Dewar, D., Lin, Z., Michalickova, K., Willems, A. R., Sassi, H., Nielsen, P. A., Rasmussen, K. J., Andersen, J. R., Johansen,, L. E., Hansen L. H., Jespersen, H., Podtelejnikov, A., Nielsen, E., Crawford, J., Poulsen, V., Sörensen, B. D., Matthiesen. J., Hendrickson, R. C., Gleeson, F., Pawson, T., Moran, M. F., Durocher, D., Mann, M., Hogue, C. W. V., Figeys, D and Tyers, M. (2002) Systematic identification of protein complexes in *Saccharomyces cerevisiae* by mass spectrometry. *Nature, 415,* 180-183.

Hazbun, T. R. and Fields, S. (2001) Networking proteins in yeast. *PNAS, 98,* 4277-4278.

Hatzimanikatis, V. and Lee, K. H. (1999) Dynamical analysis of gene networks requires both mRNA and protein expression information. *Metabolic Engineering*, ***1****(4)*, 275-281 (1999).

He, Y. D. and Friend, S. H. (2001) Microarrays-the 21st century divining rod? *Nature Medicine, 7,* 658-659.

Heath, D. (2000) *An introduction to experimental design and statistics for biology* (4th Edition) Butler & Tanner Ltd, Frome and London.

Heyer, L. J., Kruglyak. S., Yooseph, S. (1999) Exploring expression data: identification and analysis of coexpressed genes. *Genome Research, 9,* 1106-1115.

Ideker, T. E., Thorsson, V., Karp, M. R. (2000) Discovery of regulatory interactions through perturbation: inference and experimental design. *Pacific Symposium on Biocomputing, 5,* 302-313.

Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M. and Sakaki Y. (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. *PNAS, 98,* 4569-4574.

Jeong, H., Tombor, B., Albert, R., Oltvai Z. N., and Barabási, A-L. (2000) The large-scale organization of metabolic networks. *Nature, 407,* 651-654.

Jeong, H., Barabási, A-L., Tombor, B., and Oltvai Z. N., (2001a.) The global organization of cellular networks. Proceeding of *Workshop on computation of biochemical pathways and genetic networks*, Heidelberg.

Jeong, H., Mason, S. P., Barabási A-L. and Oltvai Z. N. (2001b) Lethality and centrality in protein networks. *Nature, 411,* 41-42.

Kumar, A. and Snyder, M. (2002) Protein complexes take the bait. *Nature, 415,* 123-124.

MacBeath, G. and Schreiber, S. L. (2000) Printing proteins as microarrays for high-throughput function determination. *Science, 289,* 1760-1763.

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

Sherman, F. (1998) An Introduction to the Genetics and Molecular Biology of the Yeast *Saccharomyces cerevisiae. The Encyclopedia of Molecular Biology and Molecular Medicine, 6,* 302-325.

Snyder, M., Kumar A., Agarwal, S., Heyman, J. A., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., Cheung, K-H., Miller, P., Gerstein, M. and Roeder, G. S. (2002) Subcellular localization of the yeast proteome. *Genes & Development, 16,* 707-719.

Tamayo, P., Slonim, D., Mesirov, J., Zhu, Q., Kitareewan, S., Dmitrovsky, E., Lander, E. S. and Golub, T. R. (1999) Interpreting patterns of gene expression with self-organizing maps: Methods and application to hematopoietic differentiation. *PNAS, USA 96(6),* 2907-2912.

Uetz, P., Giot, L., Cagney, G., Mansfield, T. A., Judson, R. S., Knight, J. R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S and Rothberg, J. M. (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae. Nature, 403,* 623-627.

Weaver, R. F. and Hedrick P. W. (1997). *Genetics* (2nd Edition). Wm. C. Brown Publishers, USA.

Xenarios, I. and Eisenberg, D. (2001) Protein interaction databases. *Current Opinion in Biotechnology, 12,* 334-339.

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

Zhu, H., Klemic, J. F., Chang, S., Bertone, P., Casamayor, A., Klemic, K. G., Smith, D., Gerstein, M., Reed, M. A. and Snyder, M. (2000) Analysis of yeast protein kinases using protein chips. *Nature Genetics, 26,* 283-289.

Zhu, J. and Zhang, M. Q. (2000). Cluster, function and promoter: analysis of yeast expression array. *Proceedings of Pacific Symposium on Biocomputing,* 5:476-487.

# Appendix

## Appendix 1

The 119 kinases and their cellular roles. Information is collected from the KEGG database.

**Starch and sucrose metabolism, Inostol phosphate metabolism, Sphingoglycolipid metabolism, Nicotinate and nicotinamide metabolism and Porphyrin and chlorofyll metabolism:**

| | | | | | |
|---|---|---|---|---|---|
| YAL017W | YALO18C | YBL105C | YAR018C | YBR059C | YBR160W |
| YCR091W | YCR008W | YDL079C | YDL101C | YDL108W | YDR052C |
| YDR122W | YDR477W | YDR523C | YER123W | YER129W | YGL158W |
| YGL179C | YGL180W | YGR040W | YGR052W | YHL007C | YHR030C |
| YHR079C | YHR082C | YHR102W | YHR135C | YIL095W | YJL006C |
| YJL057C | YJL106W | YJL128C | YJL141C | YJL165C | YJR059W |
| YKL048C | YKL116C | YKL126W | YKL139W | YKL161C | YKL168C |
| YKL171W | YKL198C | YLL019C | YLR096W | YLR113W | YLR248W |
| YLR362W | YML112W | YMR104C | YMR139W | YMR216C | YMR291W |
| YNL020C | YNL154C | YNL161W | YNL183C | YNL298W | YNL307C |
| YNR047W | YOL100W | YOL113W | YOL128C | YOL045W | YOR231W |
| YOR233W | YPL026C | YPL042C | YPL140C | YPL204W | YPL209C |
| YPR054W | YPR161C | YOR351C | | | |

**MAPK signalling pathway**:

| | | | | | |
|---|---|---|---|---|---|
| YBL105C | YDR052C | YGR040W | YHL007C | YHR030C | YIL147C |
| YJL095W | YJL128C | YLR113W | YLR362W | YNR031C | YOR231W |
| YPL140C | | | | | |

**Two-component system**:

YIL147C

**Second messenger signalling pathway**:

YJL164C YKL166C YPL203W

# Appendix 2

Genes encoding the proteins that interact on the protein chips.

| | | | | |
|---|---|---|---|---|
| YAL017W | YAR018C | YAR019C | YBL016W | YBL105C |
| YBR028C | YBR059C | YBR136W | YBR160W | YBR274W |
| YCL024W | YCR008W | YCR073C | YDL017W | YDL028C |
| YDL079C | YDL101C | YDL108W | YDL159W | YDR052C |
| YDR122W | YDR466W | YDR477W | YDR490C | YDR507C |
| YDR523C | YFL029C | YFR014C | YGL059W | YGL158W |
| YGL179C | YGL180W | YGR040W | YGR052W | YGR080W |
| YGR092W | YGR188C | YGR262C | YHL007C | YHR030C |
| YHR079C | YHR082C | YHR102W | YHR135C | YHR205W |
| YIL035C | YIL042C | YIL095W | YIL147C | YJL006C |
| YJL057C | YJL095W | YJL106W | YJL128C | YJL141C |
| YJL164C | YJL165C | YJL187C | YJR059W | YKL048C |
| YKL101W | YKL116C | YKL126W | YKL139W | YKL161C |
| YKL166C | YKL168C | YKL171W | YKL198C | YLR248W |
| YLR362W | YML112W | YMR001C | YMR104C | YMR139W |
| YMR216C | YMR291W | YNL020C | YNL154C | YNL161W |
| YNL183C | YNL298W | YNL307C | YNR031C | YNR031C |
| YNR047W | YOL016C | YOL016C | YOL045W | YOL061W |
| YOL100W | YOL113W | YOL128C | YOR231W | YOR233W |
| YOR267C | YOR351C | YOR351C | YOR351C | YPL026C |
| YPL031C | YPL042C | YPL140C | YPL141C | YPL150W |
| YPL153C | YPL203W | YPL204W | YPL209C | YPL236C |
| YPR054W | YPR111W | YPR161C | | |

# Appendix 3

Genes found in both the gene expression data set as well as in the data set from Schwikowski *et al.* (2000).

YAR018C
YBL016W
YBL105C
YBR059C
YBR136W
YBR160W
YBR274W
YCL024W
YDL017W
YDL028C
YDL101C
YDL108W
YDL159W
YDR052C
YDR122W
YDR477W
YDR490C
YDR507C
YFL029C
YFR014C
YGL158W
YGL180W
YGR040W
YGR080W
YGR092W
YGR188C
YHL007C
YHR030C
YHR079C
YHR102W
YHR135C
YIL035C
YIL147C
YJL006C
YJL057C
YJL095W
YJL128C
YJL141C
YJL187C
YKL116C
YKL139W
YKL166C
YLR362W
YML112W
YMR001C
YMR139W
YMR291W
YNL020C
YNL154C
YNL298W
YNL307C
YOL016C
YOL061W
YOR231W
YPL031C
YPL042C
YYPL140
YPL153C
YPL203W
YPL204W
YPR054W
YPR111W

## Appendix 4

The 17 associations that are found in the data set from Schwikowski *et al.* (2000) that are used for validating the derived protein networks is presented in the first table. Hence, these genes are the ones possible to verify in the derived protein network.

| **GENE 1** | **GENE 2** |
|---|---|
| YBL016W | YDL159W |
| YBL016W | YLR362W |
| YBR160W | YFL029C |
| YDL017W | YDR052C |
| YDL101C | YHR135C |
| YDL159W | YGR040W |
| YDR052C | YMR001C |
| YFL029C | YPR054W |
| YGR040W | YLR362W |
| YHR030C | YOR231W |
| YHR030C | YPL140C |
| YJL006C | YKL139W |
| YJL006C | YML12W |
| YJL095W | YOR231W |
| YJL095W | YPL140C |
| YJL128C | YLR362W |
| YKL139W | YML112W |

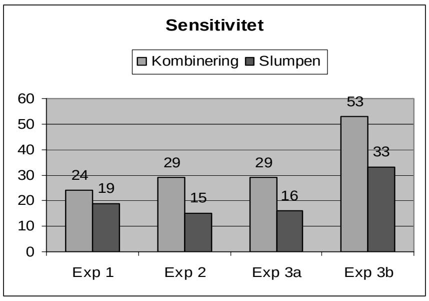
The associations found in both the data from DeRisi *et al.* (1997) and Schwikowski *et al.* (2000) are represented in the table to the left. The right table illustrates the associations found in the data sets from Chu *et al.* (1998) and Schwikowski *et al.* (2000). The cut off was set to $|0.6|$, so all the associations with a correlation over this value is represented. Gene 1 and Gene 2 are the genes that are associated and Corr stands for the calculated correlation between them.

| **GENE 1** | **GENE 2** | **CORR** |
|---|---|---|
| YGR040W | YLR362W | 0.642808 |
| YBL016W | YDL159W | 0.696635 |
| YDL159W | YGR040W | 0.755815 |
| YJL095W | YPL140C | 0.770157 |

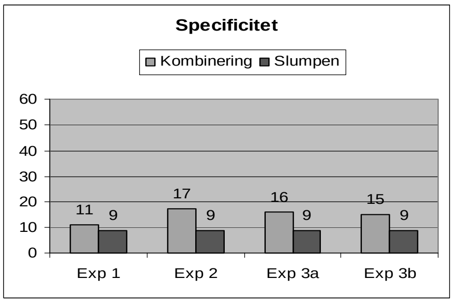
| **GENE 1** | **GENE 2** | **CORR** |
|---|---|---|
| YDL101C | YHR135C | -0.640715 |
| YHR030C | YPL140C | 0.652308 |
| YDR052C | YMR001C | 0.666662 |
| YKL139W | YML112W | 0.699926 |
| YFL029C | YPR054W | 0.811880 |

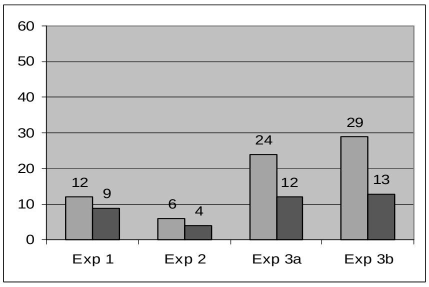
# Appendix 5

A summarize of the results in the four different experiments. The first column shows the sensitivity for the four experiments, while the second column shows the specificity for the same experiments. The first row is the sensitivity and specificity for all of the experiments when the Pearson correlation cut off was set to │0.6│, second row represents the results for cut off │0.7│, and the last row shows results when cut off was set to │0.8│.

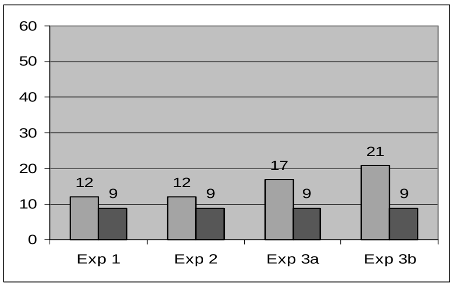


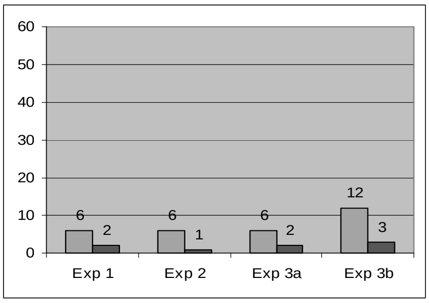
│0.6│



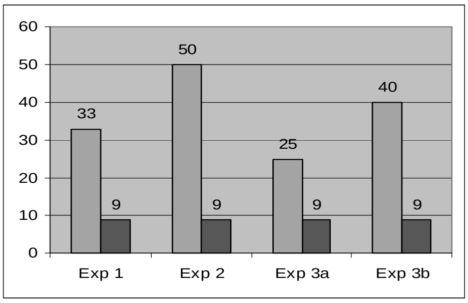


│0.7│





│0. 8│


Exp 1
Exp 2
Exp 3a
Exp 3b