ONTOLOGY-DRIVEN AND NETWORK–ENABLED SYSTEMS BIOLOGY CASE STUDIES

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ABSTRACT

Zhong Yan

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With the progress in high-throughput technologies and bioinformatics in recent years, it is possible to determine to what extent genetic or environmental manipulation of a biological system affects the expression of thousands of genes and proteins. This study requires a shift from the conventional pure hypothesis-driven approach to an integrated approach--systems biology method. Systems biology studies the relationships and interactions between various parts of a biological system. It allows individual genes or proteins to be placed in a global context of cellular functions. This analysis can answer the question of how networks of genes/proteins, differentially regulated respond to genetic or environmental modification, are placed in the global context of the protein interaction map. In this project, we establish a protein interaction network-based systems biology approach, and use the method for two case studies.

In particular, our systems biology studies consist of the following parts: (1) Analysis of mass-spectrometry derived proteomics experimental data to identify differentially expressed proteins in different genetic or environmental conditions; (2) Integration of genomics and proteomics data with experimental results, the molecular context of protein-protein interaction networks and gene functional categories; (3) Visual interpretation of molecular networks. Our approach has been validated in two case studies by comparing our discoveries with existing findings. We also obtained new insights. In the first case study, the proteomes of cisplatin-sensitive and cisplatin-resistant ovarian cancer cells were compared and we observed that cellular physiological process is significantly activated in cisplatin-resistant cell lines, and this response arises from endogenous, abiotic, and stress-related signals. We found that cisplatin-resistant cell lines demonstrated unusually high level of protein-binding activities, and a broad spectrum of across-the-board drug-binding and nucleotide-binding mechanisms are all activated. In the second case study, we found that the significantly enriched GO categories included genes that are related to Grr1 perturbation induced morphological phenotype change are highly connected in the GO sub-network, which implies that Grr1 could be affecting this process by affecting a small core group of proteins. These biological discoveries support the significance of developing a common framework of evaluating functional genomics and proteomics data, using networks and systems approaches.

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1. INTRODUCTION

Over the past two centuries, life science research has been rooted in the assumption that complex problems may be solvable by dividing them into smaller, simpler, and thus more manageable units. While the human body is considered to be an integrated system with a company of components, the natural tendency of medicine is to separate the single factor that is most responsible for the consequence. It is undeniable that this approach has been a success for years. However, it leaves little room for contextual information. The need to make sense of complex interactions has led some researchers to shift from a component-level to system-level perspective. With the progress in high-throughput technologies and bioinformatics (for example, many bioinformatics databases are available to the public) in recent years, it is possible to determine to what extent genetic or environmental manipulation of a biological system affects the expression of thousands of genes and proteins. This form of study requires a shift from a conventional individual approach (divide-and-conquer approach) towards an integrated approach. The integrated approach leads to an emerging field called systems biology[1]. Systems biology takes into account complex interactions of genes, proteins, and cell elements. By studying the relationships and interactions between various parts of a biological system, it is hoped that researchers might build a system-level understanding of biological systems and gain novel insights towards discoveries.

In this project, we have developed a novel systems biology approach to study proteomics experimental data. Using this approach we have performed case studies on two proteomics datasets: (1) human ovarian cancer drug resistance; (2) yeast Grr1 knock-out. Our systems biology studies consist of the following parts (see figure 1.1). (1) Analyzing mass-spectrometry derived proteomics experimental data to identify differentially expressed proteins in cisplatin-sensitive vs. cisplatin-resistant ovarian cell line samples and yeast Grr1 knock-out vs. wild-type samples; (2) Integrating genomics and functional genomics data with experimental results and the molecular context of protein-protein interaction networks and gene functional categories: we use OPHID (Online Predicted Human Interaction Database) for our ovarian cancer study and an in-house developed yeast protein-protein interaction database (SBG) for our yeast study. The integration involves identifying protein interaction partners for the differentially-expressed protein set ("seed proteins"), as well as identifying the gene ontology cross-talk partners in the context of the protein-protein interaction network; (3) Visual interpretation of molecular networks.[2]

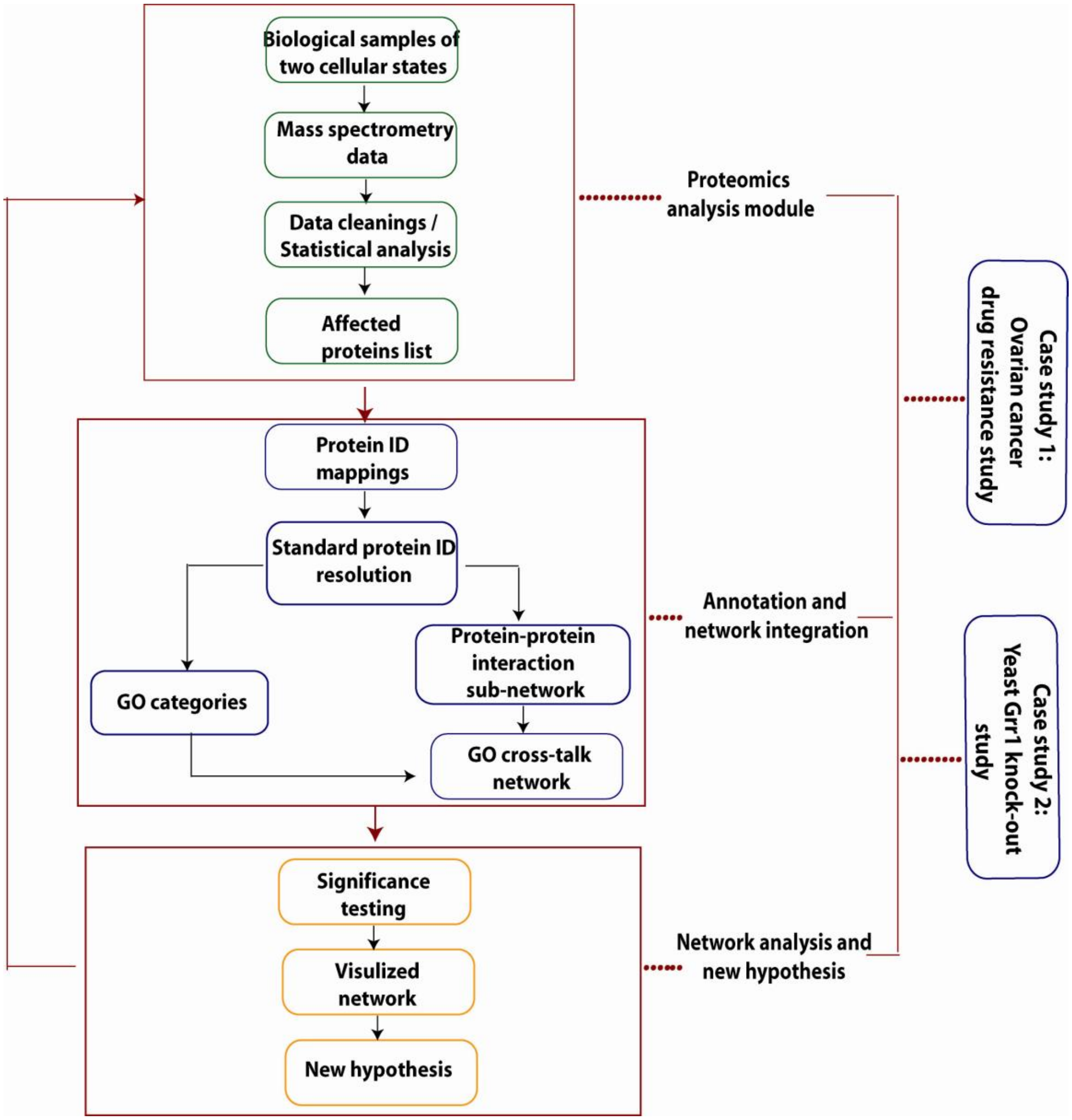

Biological samples of two cellular states
Mass spectrometry data
Data cleanings / Statistical analysis
Affected proteins list
Proteomics analysis module
Protein ID mappings
Standard protein ID resolution
Protein-protein interaction sub-network
GO categories
GO cross-talk network
Annotation and network integration
Significance testing
Visulized network
New hypothesis
Network analysis and new hypothesis
Case study 1: Ovarian cancer drug resistance study
Case study 2: Yeast Grr1 knock-out study

Figure 1.1 Framework of novel systems biology approach

Unlike conventional methods, which lack functional integration of data and effective analysis tools to derive functional relationships between heterogeneous while related data, our studies have the following significance. First, we have developed a novel systems biology approach which can identify “significantly interacting protein categories”. This is distinct from the recent approach of using GO annotations for differentially expressed gene classifications resulting from microarray analysis[3]. Our method can be generalized to enable other similar systems biology studies, in which statistically significant experimental “omics” results, public protein interactome data, and genome/proteome annotation database are integrated into an easy-to-interpret two-dimensional visualization matrix[2]. Second, to integrate yeast protein-protein interaction data from different sources, we have created our own metadata for experimental methods that are used to detect interacting protein pairs (see section 3.2 in paragraph “Protein Interactome Data”). Third, we have developed our unique scoring model (see section 3.3) to calculate reliability scores for the interacting protein pairs. We applied our scoring model to the combined protein-protein interaction dataset to calculate a reliability score for each unique interacting pair. This enables our significant protein ranking analysis (see section 3.10). Fourth, we applied a unique molecular network visual representation scheme to the significant biological process categories and significant between-category interactions (see section 3.5 and section 4. for two case studies). Our new approach based analysis will help the life science researchers validate their discoveries and generate new hypotheses.

2. BACKGROUND

2.1 Mass Spectrometry - based Proteomics

Proteomics refers to the branch of discovery science focusing on large scale analysis of proteins. Initially, the term proteomics was used to describe the study of expressed proteins of a genome using a combination of two-dimensional (2D) gel electrophoresis to separate proteins and mass spectrometry (MS) to identify them. This approach is now referred to as “expression” or “global profiling” proteomics. However, the scope of proteomics has now broadened to include the study of “protein-protein” interactions (protein complexes), referred to as cell-mapping proteomics [4, 5]. Proteomics complements other functional genomics, including microarray expression profiling, systematic phenotypic profiling, systematic genetics, and small-molecule-based arrays [6]. Compared with genomics, proteomics is much more complicated. While the genome is rather stable, the proteome differs from cell to cell and is constantly changing through its biochemical interactions with the genome and the environment.

Mass spectrometry-based proteomics has a distinct application in unraveling the levels of protein abundance, post-translational modifications (e.g., glycosylation, acetylation, phosphorylation, and myristoylation), as well as protein-protein interactions, which are the formative drive in a cell. Changes in these parameters are not revealed by measuring mRNA levels. Mass spectrometry-based proteomics provides opportunities to identify target proteins that are differentially regulated under different conditions. It helps biologists elucidate the dynamics of important signaling and regulatory networks in biological process.

2.1.1 Mass spectrometry

Mass spectrometry is the method for determining the molecular weight of chemical compounds by separating molecular ions according to their mass-to-charge ratio (m/z). Mass spectrometers are powerful devices used for this purpose. Mass spectrometric measurements are carried out in the gas phase on ionized analytes. A mass spectrometer consists of an ionization source for ion-generation, a mass analyzer that measures the mass-to-charge ratio (m/z) of the ionized analytes, and a detector that registers the number of ions at each m/z value. The ionization source transfers molecules from solution or solid phase into gas-phase ions that can then be manipulated within electric or magnetic fields. Ionization techniques are critical for determining what types of samples can be analyzed by mass spectrometry. The two most frequently used ionization techniques are ESI (Electrospray Ionization) and MALDI (Matrix-Assisted Laser Desorption/Ionization). ESI ionizes the analytes out of a solution and is therefore readily coupled to liquid-based separation tools such as HPLC. MALDI sublimates and ionizes the samples out of a fry, crystalline matrix via laser pulses. MALDI-MS is normally used to analyze relatively simple peptide mixtures, whereas integrated liquid-chromatography ESI-MS systems (LC-MS) are preferred for the analysis of complex samples [7, 8].

The mass-analyzer is used to separate gas-phase ions based on their mass-to-charge (m/z) ratios, and is central to the technology. In the context of proteomics, its key parameters are sensitivity, resolution, mass accuracy and its ability to generate information-rich ion mass spectra from peptide fragments (tandem mass or MS-MS spectra). There are four basic types of mass analyzers currently used in proteomics research. These are the ion trap, time-of-flight (TOF), quadrupole, and Fourier Transform ion cyclotron (FT-ICR-MS) analyzers. They are very different in design and performance and, each has its own strength and weakness. These analyzers can be stand alone or, in some cases, put in tandem to take advantage of the strengths of each [8-11].

Both MALDI and ESI are soft ionization techniques in that ions are created with low internal energy and thus undergo little fragmentation. Mass-to-charge ratios can be readily and accurately measured for intact ions, but this information does not provide data on the covalent structure of the ion. For peptides and proteins in particular, data related to the amino acid sequence of the molecule are desired. To generate this information, new configurations of mass spectrometers have been developed to isolate ions, fragment them, and then measure the mass-to-charge ratio of the fragments. These devices are collectively called tandem mass spectrometers. A tandem mass spectrometer is a mass spectrometer that has more than one analyzer, in practice usually two. The two analyzers are separated by a collision cell into which an inert gas (e.g. argon, henium) is admitted to collide with the selected sample ions and bring about their fragmentation (collision-induced dissociation or CID). The analyzers can be of the same or different types, the most combinations being quadrupole-quadrupole, magnetic sector-quadrupole, and quadrupole-TOF [12, 13].

The first analyzer of a tandem mass spectrometer is used to select user-specific peptide ions from peptide mixtures. These chosen ions then pass into the collision cell, and are bombarded by the gas molecules into fragment ions, which are then analyzed. The original mass to charge ratio of each ion as well its specific fragment spectrum are used to search a database of theoretical peptide fragmentation spectra often resulting in unambiguous peptide identification. The data from each of these methodologies is represented as output peak list files adherent to a specific file format that is dependent on the instrument used for analysis. Programs such as SEQUEST [14] and MASCOT (http//www.matrixscience.com) correlate the experimentally acquired MS/MS spectra to the computer generated MS/MS spectra and produce various scores used to assess the validity of this correlation. Each correlation program uses a different algorithm to assign peptides and thus each program produces overlapping but variable outputs. Various laboratories have used different approaches to exploit the advantages of both software algorithms [15] and to validate more thoroughly the results of these algorithms individually [16, 17]. It is apparent that no single analysis system has been universally accepted to date.

2.1.2 Proteomics data analysis

In a typical mass spectrometry based experiment, protein samples are digested by a protease (usually trypsin) and the resulting peptides can be further separated by liquid chromatography before directly introduced into MS. The peptide fragment masses are determined by MS, which provides a fingerprint of the protein of interest. The masses are compared to the predicted proteolytic peptides from sequence databases taking into account user specified parameters such as the number of missed cleavage sites. If, however, database searching leads to ambiguous results, then further MS analyses, involving the usage of tandem mass spectrometry (MS/MS), are undertaken sequentially on each peptide in the mixture to generate a sequence, or partial sequence, known as a sequence tag, for these peptides. This is frequently achieved by using ESI-MS/MS. Further database searching with both the molecular mass of the peptide and the sequence tag information should lead to unambiguous protein identification[18-20]. Finally, the instrument generates an output peak list file in a specific file format depending on the type of instrument used, and an analysis pipeline (Figure 2.1) can be used to take the peak list file as input and generate a series of output files.

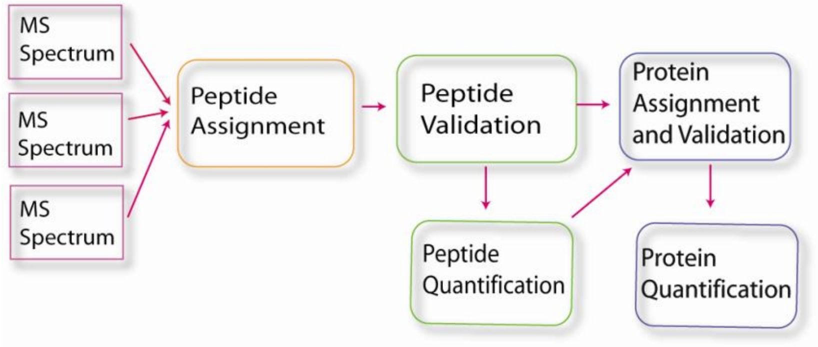


Figure 2.1 Proteomics data analysis pipeline

2.1.3 Proteomics data management tools

The scale and complexity of proteomics data require software tools to facilitate data management. Compared with microarray data management tools, there are few tools available for mass spectrometry proteomics studies. Below we summarize most of the proteomics data management tools. This work is based on my previous publication [21].

PEDRo database tool (http://pedro.man.ac.uk) is an open source tool for proteomics data entry and modeling. However, it is not a comprehensive query and analysis tool. The PEDRo tool implements the PEDRo data model (Refer to section 3) which was released early in 2003. The schema of the PEDRo data model is available at the website. PEDRo supports an ontology service. It stores the XML directly in an open-source XML storage system, Xindice. The data are presented to the users by gathering web pages from the stored XML using XSLT.[22, 23]

SBEAMS-Proteomics (http://www.sbeams.org/Proteomics/) is one of the modules of SBEAMS integrated platform developed by ISB that is used for proteomics experimental data storage and retrieval. These experiments can be correlated later under the same framework. The integrated open source system SBEAMS adopts a relational database management system backend and a web interface front end. Information about the quality of identification can be stored with the data; peptides which could not be properly identified from mass spectra can be flagged and reanalyzed with additional searches. The database schema for SBEAMS-Proteomics is available at the website (http://www.sbeams.org/Proteomics/).

ProteinScape is a commercial client-server platform for proteomics data management (http://www.bdal.com/proteinscape.html). It organizes data such as gel data, mass spectra, process parameters, and search results. It can manage gel-based or LC-based workflows, as well as quantitative proteomics. ProteinScape also enables automated analysis through interactions with database search engines such as Mascot, Phenux, and Profound. ProteinScape's relational database system can be Microsoft SQL or Oracle 9.1.

PROTEIOS (http://www.proteios.org/) is an mzData-compliant open source client-server application that implements mass spectrometry data storage, organization, and annotation. The server is a relational database that can be MySQL, Oracle, as well as utilize other alternatives. The client side runs as a Java application. One of the main objectives of Proteios is to provide a GUI enabling queries based on experiment data and annotation data. The schematic diagram is available at the website. Currently the input data files must be in XML format. It is working on imports of tab-separated files [24].

PROTICdb is a web-based proteomics data management tool used for plant proteomics data storage and analysis. The data can come from 2D-GEL and MS. The data stored can also be in the form of quantitative measurements. To support data interpretation, PROTICdb allows the integration of information from the user's own expertise and other sources into a knowledge base. It also provides links to external databases [25].

ProDB is an open source proteomics data management tool (http://www.cebitec.uni-bielefeld.de/groups/brf/software/prodb_info/index.html) that can handle data conversion between different mass spectrometer software, automate data analysis, and allow the annotation of MS spectra (i.e. assigning gene names or storing data on protein modifications). The system is based on an extensive relational database to store the mass spectra together with the experimental setup [26]. The first release will be available to the public soon.

There are several other proteomics data management tools not described here, such as PROTEUS [27], Proteomics Rims (developed by Bruker BioSciences), Xome and Mass Navigator [28].

2.2 Ontology-based Gene Annotation and Network-enabled Analysis

2.2.1 Proteomics and Systems Biology

The goal of proteomics research is to understand the expression and function of proteins on a global level. It strives to characterize protein structure and function, protein-protein, protein-nucleic acid, protein-lipid, and enzyme-substrate interactions, post-translational modifications, protein processing and folding, protein activation, cellular and sub-cellular localization, protein turnover and synthesis rates, and even alternative isoforms caused by differential splicing and promoter usage. In addition, the ability to capture and compare all of this information between two cellular states is essential for understanding cellular responses. Achieving the goals of proteomics is not trivial. Adding to the complexity of this field is the need to integrate proteomics data with other information to fully understand how systems work.

Systems biology is a newly emerging field that seeks to analyze the relationships among elements in a system in response to genetic or environmental perturbations, with the goal of understanding the system or the properties of the system[29]. Therefore, systems biology is a holistic approach that seeks to integrate biological data as an attempt to understand how biological systems function, thus being distinct from a pure omics - based or other bioinformatics methods. The present thesis is an attempt in this direction. We captured the proteome difference between cellular states, and integrate this information with information from gene ontology as well as protein interaction database. Thus, for the first time, it provides an in-depth interpretation at the molecular signaling network level.

In particular, our systems biology approach consists of the following three major elements [2]: (1) Omics: analyzing mass-spectrometry derived proteomics experimental data to identify differentially expressed proteins in different genetic or environmental conditions; (2) Ontology: annotating the proteomics data based on gene ontology functional categories; (3) Network: mapping the proteomic data into protein-protein interaction network and translating the protein-protein interaction network into a gene ontology cross-talk network.

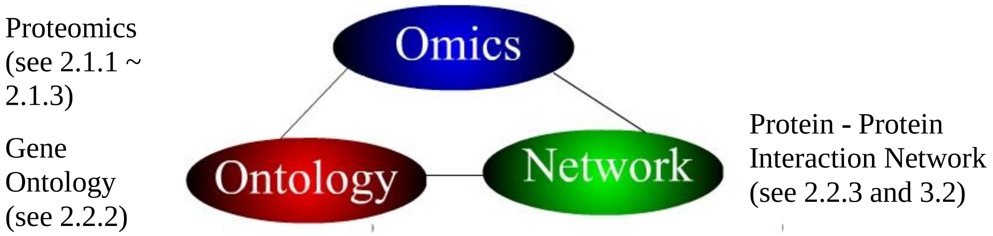


Figure 2.2 Major elements of our systems biology approach. The lines represent the tight connections of the elements.

Figure 2.2 shows the three major elements of our systems biological approach. In this approach, the data from omics experimental results is analyzed against gene functional categories and gene functional category network. It is the first time that the gene ontology concept has been brought to the molecular context of protein-protein interaction networks, which has been used to interpret the proteomics experimental result.

2.2.2 Ontology – based gene annotations

From the point of view of systems biology, the interpretation of differentially expressed protein lists identified from proteomics experiments is not a trivial task. Given a set of differentially expressed genes / proteins, or a set of genes / proteins in a cluster, one would often wish to know whether these genes / proteins share a common function, subcellular localization, metabolic or regulatory pathway. In addition to characterizing the gene/protein set, this type of analysis may also reveal information on new and previously unknown genes in the set. This type of work often requires the mapping of the genes/proteins into gene ontology (GO) terms. The introduction of Gene Ontology (GO) as a standardized vocabulary for describing genes, gene products and their biological functions represents an important milestone in the possibilities to handle and include biological background information in functional genomics and proteomics analyses.

The gene ontology is represented as a network, or a ‘directed acyclic graph’ (DAG), in which terms may have multiple parents and multiple relationships to their parents. The controlled vocabularies are structured in levels so that attributes can be assigned to a gene product at different levels of description, depending on how much is known about this gene product.[30] There are three different sets of vocabularies for gene ontology: (1) Molecular function describes the activity of a gene product at the molecular level. It does not provide information about the compounds or locations of the activity. Example of molecular function at level 2 can be binding and at level 3 can be protein binding. The more specific term at level 4 can be transcription factor binding. (2) Biological process describes recognized series of events or molecular functions. A biological process is not equivalent to a pathway though some GO terms do describe pathways. Examples of biological process are death at level 3 and cell death at level 4. (3) Cellular component refers to the location in the cell in which a gene product exerts its activity. Examples are nucleolus, organelle, and polarisome. Many databases today provide GO annotations for a variety of organisms including humans, yeast, and other species.

Annotation of genes with GO terms creates a biological knowledge profile in three layers (biological process, molecular function, or cellular component). Three common methods are used to query GO categories: by individual gene, by gene function, and by using a list of genes [31]. Translation of the differentially expressed gene/protein list into a functional profile helps biologist get insight into the cellular mechanisms relevant to a given condition. Therefore, it has been widely used in the analysis of functional genomics and proteomics studies [32-36].

The ontological analysis of gene expression or proteomics data usually follows the following steps: (1) Prepare the gene or protein list of interest. (2) Prepare reference gene or protein list which is used to calculate P-values against. (3) Map both lists (interested list and reference list) to GO categories. (4) Select statistical model. The gene ontology analysis can be performed with a number of statistical models including hypergeometric, binomial, Fisher’s exact test, and chi-square. These tests are discussed in detail in [37]. (5) Find significantly enriched GO categories in your list of interest using the selected statistical model. (6) Perform corrections for multiple comparisons. When many genes/proteins are analyzed at the same time, some significance will happen by chance. The multiple comparison corrections control the overall probability of making a Type I error. Many different statistical methods have been published to perform this kind of correction, for example, Bonferroni correction [38], FDR [39], and permutation correction [40]. Each method has its unique feature. (7) Interpret the result in terms of the biological significance.

The statistical evaluation of enriched GO categories enables to highlight the most significant biological characteristics of a gene or protein set, therefore allows us to mine knowledge from data. In recent years, many tools have been developed to automate the gene ontology analysis. Table 2.1 lists some popular GO analysis tools, the statistical models the tools used, multiple comparison correction methods implemented, and the GO visualization view.

Table 2.1 Gene Ontology analysis tools

Tool	Statistical model	Multiple comparison corrections	GO visualization
WebGestalt[41]	Hypergeometric test, Fisher's exact test	NA	Tree, bar chart, DAG
GeneMerge[42]	Hypergeometric	Bonferroni	No tree view
CLENCH[43]	Chi-square test, Binomial, Hypergeometric	NA	DAG
GOSurfer[44]	Chi-square test	FDR	Tree
Onto-Express[45]	Chi-square test, Binomial, Hypergeometric, Fisher's exact test	Bonferroni, Holm, Sidak	Tree
GOToolBox[46]	Binomial, Hypergeometric, Fisher's exact test	Bonferroni, Holm,FDR, Hochberg, Hommel,	NA
Onto-Express[45, 47]	Binomial, Hypergeometric, Chi-square test	Bonferroni, Holm, Sidak, FDR	Tree, bar chart
GO Term Finder[48]	Binomial	NA	Tree

The ontology-based omics data analysis approach enables researchers to find out enriched functional categories involved in the experimental conditions. While biological systems contain large number of different genes and proteins that are interacted with each other, it is necessary to develop an approach to bring the ontology-based omics data analysis to the interaction network. This integrated approach will definitely benefit the biologists to obtain more insight for biological phenomena.

2.2.3 Protein interaction network- based analysis

A discrete biological function is rarely attributable to an individual protein [49]. Instead, most biological characteristics arise from complex interactions between the cell's numerous constituents, such as proteins, DNA, RNA and small molecules. In particular, the network of protein-protein interactions, also referred to as interactome, forms a backbone of signaling pathways, metabolic pathways and cellular processes for normal cell function. Protein interaction network analysis provides an effective way to understand the relationships between genes. It places the genes identified in functional genomics and proteomics experiments in a broader biological context, thereby facilitating the understanding of the structure and function of a living cell.

The network-based analysis has been enabled by the recent elucidation of large-scale protein interaction networks in different species, including *S. cerevisiae* (yeast)[50-53], *D. melanogaster* (fly)[54], *C. elegans* (worm)[55] and *H. sapiens* (human)[56, 57].)[56, 57]. Collections of these protein interactions, representing a subset of the whole interactome, are stored in various data repositories as the Database of Interacting Proteins (DIP) (ref), the Biomolecular Interaction Database (BIND), The Molecular INTeraction Database (MINT), InAct, the human Protein Reference Database (HPRD) and the Online Predicted Human Interaction Database (OPHID) [58]. The protein network-based analysis has been utilized for the analysis of functional genomics experiments recently [59, 60].

The most comprehensive database for a human protein network is the Online Predicted Human Interaction Database (OPHID) [58]. OPHID is a web-based database of predicted interactions between human proteins. It combines the literature-derived human protein-protein interactions from BIND (Biomolecular Interaction Network

Database), HPRD (Human Protein Reference Database) and MINT (Molecular Interactions Database), with predictions made from *Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster* and *Mus musculus*. OPHID catalogs 16034 known human PPIs obtained from BIND, MINT and HPRD, and makes predictions for 23889 additional interactions.[58] It is designed to be both a resource for the laboratory scientist to explore known and predicted protein-protein interactions, and to facilitate bioinformatics initiatives exploring protein interaction networks. It should be noted that OPHID predicted human interaction are hypothetical and are likely to have some false positives as well as missing protein interactions. However, it was claimed that approximately half of the predicted interactions using interlogs between microorganisms can be experimentally validated [61].

Table 2.2 Leading protein interaction databases

Name	**Description**
Online Predicted Human Interaction (OPHID) http://ophid.utoronto.ca/ophid/	Information about known human PPIs from Database BIND, MINT, and HPRD, as well as large number of predicted human PPIs
Human Protein Reference Database (HPRD) http://www.hprd.org/	Manually curated and extracted from literature for human PPIs
Saccharomyces Genome Database (SGD) http://www.yeastgenome.org/	Comprehensive database that contains genetic and physical interactions for yeast proteins. More than 90% interactions come from GRID
General Repository for Interaction Datasets (GRID) http://biodata.mshri.on.ca/grid	Genetic and physical interactions for yeast, fly, and worm proteins. Interactions data comes from literature, BIND, and MIPS, including several genome/proteome-wide studies
Biomolecular Interaction Network Database (BIND) http://www.isc.org/index.pl?/sw/bind/	Physical, biochemical, genetic interactions, and interactions between DNA, RNA, proteins, small molecules, including interactions from human, yeast, mouse, rat, and many other species.
Human Annotated Protein Protein Interaction (HAPPI) http://bio.informatics.iupui.edu/HAPPI/index.stm	Database that contains protein interactions from String, OPHID, and HPRD.

On the other hand, SGD (Saccharomyces Genome Database) is a scientific database of the molecular biology and genetics of the yeast *Saccharomyces cerevisiae,* which is commonly known as Baker's or budding yeast. Besides protein-protein interaction datasets, SGD also contains genes and proteins sequence information, descriptions and classifications of their biological roles, molecular functions, and subcellular localizations, and links to literature information (see table 2.2) [62-64]. More than 90% of the interactions stored in SGD come from GRID. BIND (Biomolecular Interaction Network Database) is the database that stores the interactions between DNA, RNA, proteins, and small molecules for many species including yeast [65-67]. Table 2.2 lists the information for the leading protein interaction databases.

The protein network-based analysis has been considered as one of the most important elements of the systems biology approach. Protein network analysis place the genes identified in microarray experiments or differentially expressed proteins detected in mass-spectrometry experiments in a global biological context. Protein-protein interaction networks reflect the functional grouping of these coordinated genes/proteins. It enables the study of the roles of subsets of genes/proteins.

A few papers published recently reported mapping the differentially expressed protein lists identified through microarray or proteomics experiments into protein-protein interaction database such as OPHID. Using the network-based analysis, Wachi et al found that the genes differentially elevated in cancer, as obtained from microarray profiling data, are well connected[60]. In this study, genes in the array were mapped onto OPHID using gene symbols and protein sequences. Connectivity analysis was performed for the protein network constructed. Then k-core analysis was conducted, where less connected nodes were removed in an iterative way. This resulted in a series of subgraphs that gradually revealed the globally central region of the original network. Using k-core analysis, the authors measured how differentially expressed genes were close to the topological center of the protein network. Centrality of the genes is associated with the essential functions of the genes in the yeast. The analysis concluded that squamous cell lung cancer genes share similar topological features for essential proteins.

Calvano et al recently performed a network analysis of systematic inflammation in humans [68]. Gene expression patterns in human leukocytes receiving an inflammatory stimulus were first analyzed using genome-wide microarray analysis. Genes significantly perturbed after stimulus were identified using significance analysis of microarray method, which controls the false discovery rate to less than 0.1%. To identify significant pathways in a biological process, the differentially expressed genes were overlaid onto the interactome, the Ingenuity Pathways Knowledge Base (KB), which is the largest curated database of previously published findings on mammalian biology from the public literature. Target genes were identified as the subset having direct interactions with other genes in the database. The specificity of connections for each target gene was calculated by the percentage of its connections to other significant genes. Pathways of highly connected genes were identified by likelihood. Using this strategy, the authors demonstrated that, upon acute systematic inflammation, the human blood leukocyte response includes widespread suppression at the transcriptional level of mitochondria energy production and protein synthesis machinery.

Said, et al [69] used protein interaction networks to analyze the phenotypic effects in yeast. Toxicity-modulation, non-phenotypic classifications, and high-throughput genomic phenotyping were conducted. Networks that represented a phenotypically annotated interactome of essential, toxicity-modulating, and no-phenotype proteins were constructed. The analysis showed interesting results. For example, toxicologically important protein complexes, pathways, and modules were identified, which have potential implications for understanding toxicity-modulating processes relevant to human diseases.

In other studies, Seiden-Long et al integrated the microarray datasets with OPHID and found six of the target genes by HGF/Met/RAS signaling belong to a hypothetical network of function at the protein level [70]. Motamed-Khorasani et al found that six of the total of 17 androgen-regulated genes could be mapped into OPHID database. Five of the six genes are networked within two interacting partners [71].

The current project will integrate the three elements: proteomics, ontology, and network, and perform ontology-driven and network-enabled systems biology case studies. The following sections will describe the details of our methods and results.

3. METHODS

All methods related to ovarian cancer study in this section were based on the published methods and private communications with Dr. Chen, Dr. Shen, and Dr. Wang. I am one of the primary contributing members (Chen, J., Yan, Y., Shen, C., Fitzpatrick, D., Wang, M. A Systems Biology Case Study of Ovarian Cancer Drug Resistance. JBCB, 2007[2].). Method in 3.2.2 was kindly provided by Dr. Shen, who developed a statistical model to identify differentially expressed proteins as one of the inputs of my case study 2. Ovarian cancer proteomics experimental methods in section 3.1.1 were kindly provided by Dr. Mu Wang. Yeast proteomics experimental methods in section 3.1.2 were kindly provided by Josh Heyen. Tables 3.1 ~ 3.3 were based on discussion with Dr. Goebl and Josh Heyen, where I am one of the contributors. The use of the materials was granted with the permission from participating contributors.

3.1 Proteomics Method

3.1.1 Ovarian cancer drug resistance proteomics method

A2780 and 2008 cisplatin-sensitive human ovarian cancer cell lines and their resistant counterparts, A2780/CP and 2008/C13*5.25, were used in the ovarian cancer drug resistant study. Proteins were prepared and subjected to LC/MS/MS analysis as described in [72]. There were two groups (two different parent cell lines), six samples per cell line, and two HPLC injections per sample. Samples were run on a Surveyor HPLC (ThermoFinnigan) with a C18 microbore column (Zorbax 300SB-C18, 1mm x 5cm). All tryptic peptides (100 μL or 20 μg) were injected onto the column in random order. Peptides were eluted with a linear gradient from 5 to 45% acetonitrile developed over 120 min at a flow rate of 50 μL/min. Fluant was introduced into a ThermoFinnigan LTQ linear ion-trap mass spectrometer. The data were collected in the “triple-play” mode (MS scan, Zoom scan, and MS/MS scan). The acquired data were filtered by proprietary software and Database searching against International Protein Index (IPI) database. NR-Homo Sapiens database was carried out using both SEQUEST and X!Tandem algorithms. Protein quantification was carried out using the

LC/MS-based label-free proprietary protein quantification software licensed from Eli Lilly and Company [72]. Briefly, once raw files are acquired from the LTQ, all extracted ion chromatogram (XIC) is aligned by retention time. Each aligned peak should match parent ion, charge state, daughter ions (MS/MS data) and retention time (within a one-minute window). If any of these parameters were not matched, the peak will be disqualified from the quantification analysis. The area-under-the-curve (AUC) from individually aligned peak was measured, normalized, and compared for their relative abundance. All peak intensities were transformed to a $\log_2$ scale before quantile normalization [73]. If multiple peptides have the same protein identification, then their quantile normalized $\log_2$ intensities were averaged to obtain $\log_2$ protein intensities. The $\log_2$ protein intensity is the final quantity that is fit by a separate ANOVA statistical model for each protein. $\log_2$ (Intensity) = overall mean + group effect (fixed) + sample effect (random) + replicate effect (random). Group effect refers to the effect caused by the experimental conditions or treatments being evaluated. Sample effect is caused by random effects from individual biological samples. It also includes random effects from sample preparation. The replicate effect refers to the random effects from replicate injections of the same sample. All of the injections were in random order and the instrument was operated by the same operator. The inverse $\log_2$ of each sample mean was determined to resolve the fold change between samples.

3.1.2 Yeast Grr1 knock-out proteomics method

For the yeast Grr1 knock-out study, a customized SILAC approach was used to perform mass labeling. *S.cerevisiae* strain DBY2059 (Mat α leu2-3) was cultured overnight to stationary phase in two replicate 10ml batches of modified SD media consisting of 2% glucose, .5% glutamine, and .05 mg/ml C_6^{13} leucine (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). Concurrently, strain JH001 (Mat A, grr1Δ::Nat) was also cultured overnight to stationary phase in two replicate 10ml batches of the same media supplemented with C_6^{12} leucine. Each 10ml culture was then used to inoculate a 500ml culture of the same media and cells were grown for nine population doublings to mid-log phase (~5x10^6 cells/ml). Cell density was determined by cell counting using a hemacytometer (Reichert, Buffalo,NY, USA.).

Cells were harvested by centrifugation in a Beckman JA-14 rotor at 4000 X G for 10 minutes, washed three times in ice cold water, and immediately re-suspended in 5ml of extraction buffer [8M Urea, 0.1M Ammonium Bicarbonate]. Cells were then immediately flash frozen in liquid nitrogen and stored at -80C overnight. Protein extract was prepared the following day by manual bead beating using 300 um acid washed glass beads (Sigma, St.Louis, MO). Specifically, samples were subjected to 10 cycles consisting of 30 seconds on ice and 30 seconds of vortexing in the presence of glass beads. Glass beads and cellular debris were then spun down at 2000 X G and the supernatant was placed in 15ml conical tubes. Protein concentrations were determined using the Bradford protein assay and protein samples were mixed in a 1:1 ratio (DBY2059 C_6^{13} leucine: JH001 C_6^{12} leucine) producing two replicate protein mixes from four independently grown batch cultures. Each protein mixture was diluted with 100mM Ammonium Bicarbonate to a final Urea concentration of 4M. Protein disulfide bond reduction was carried out by adding a 40 fold molar excess of Dithiothreitol (DTT) to each protein mixture followed by a three hour incubation at 36°C. Reduced protein mixtures were then alkylated using a 1:80 molar ratio of protein to iodoacetamide (IAM) followed by incubation on ice in complete darkness for 2 hours. The reduced and alkylated protein mixture was then diluted to 2M Urea using an equal volume of 100mM ammonium bicarbonate and subjected to trypsin digestion using 2% (weight/weight) of TPCK-treated trypsin. Digestion was carried out at 37°C for twenty four hours. Peptide samples were then dried down in a speed-vac and resuspended in a buffer consisting of 5% Acetonitrile, 95% EMD water, 0.025% Formic Acid, and 0.0025% HFBA.

The two replicate peptide mixtures were analyzed 3 times each through an automated de-salt/2DLC/MS system. Peptide De-salting and separation were performed in tandem using the Paradigm MG4 HPLC System (Michrom Biosciences, Inc.). Initially, approximately 150ug of the tryptic peptide mixture was loaded directly onto a C-18 microtrap (Michrom Biosciences, Inc.) and desalted by flushing the trap with 20 column volumes of mobile phase A (2% Acetonitrile, 98% Water, 0.025% Formic Acid) at a flow rate of 50 ul/min. Peptides were then eluted onto an SCX microtrap (Michrom Biosciences, Inc.) using 20 volumes of mobile phase B (98% Acetonitrile, 2% Water, 0.025% Formic Acid, 0.001% HFBA). Peptides were then bumped off the SCX microtrap in a stepwise fashion using increasing concentrations of Ammonium Formate. Ten steps were used in our analysis of 0, 4, 8, 12, 15, 18, 21, 25, 50, and 100 mM Ammonium Formate followed by two identical steps of 1M Ammonium Formate. Each population of peptides were eluted off the SCX micro-trap onto a C8 nano-trap (Michrom Biosciences, Inc.) coupled directly to a hand packed C18 column with a hand pulled tip. A home made high pressure bomb was used to pack 15 cm of 5um-100 angstrom Magic C18 resin (Michrom Biosciences, Inc.). Peptides were then eluted off this column at 500nl/min using an Acetonitrile gradient from 5-50% and analyzed by an LTQ Mass Spectrometer (Thermo Electron Corporation) on the fly.

The LTQ-MS was set for data dependent MS/MS acquisition with a total ion count threshold of 1000. Dynamic exclusion was used to only collect two MS/MS spectra on a single parent ion every 45 seconds. Two types of data collection were performed in this analysis termed gas phase fractionation and full scan analysis. Typically, the LTQ-MS is set to scan across an m/z range from 500-2000 throughout the course of the analysis. This type of analysis was done in replicate for both replicate peptide mixtures culminating in four, 12 step full scan analyses. Each of the peptide mixtures was also subjected to a single gas phase fractionation analysis. This analysis is essentially equivalent to three full scan analyses but the mass spectrometer is set to scan 1/3 of the m/z scan range. This allows for greater m/z resolution and increased peptide detection sensitivity due to the fact MS/MS spectra are being collected for a smaller fraction of the peptide population eluting from the column. However, this process is time consuming given that three separate analyses must be performed to acquire data across the whole scan range and thus we only conducted a single gas phase analysis for each peptide mixture. The scan ranges for gas phase fractionation were 500-1000 m/z, 900-1500 m/z, and 1400-2000 m/z. In all, each of the two replicate peptide mixes were loaded and analyzed five times through the 2D-LC-MS system for a total of ten different runs.

Peptide assignments for experimental MS/MS spectra were made using the SEQUEST program (Thermo Electron Corporation). The 12 raw files generated for each run are run individually through the SEQUEST software. Peptide assignments were then analyzed for validity using a suite of software available from the Institute for Systems Biology termed the Trans-Proteomic Pipeline. This analysis toolkit provides computational tools that validate peptide assignments (Peptide Prophet), protein assignments (Protein Prophet), and quantify relative peptide and protein abundance ratios (ASAPRatio). It is important to note that prior to analysis through the TPP the 12 .raw files are combined into a single mzXML using the TPP. This mzXML file captures raw parent MS spectra for use in quantification by the program, ASAPratio. The SEQUEST output files are converted to summary.html files that are readable by the programs Peptide Prophet and Protein Prophet. All the individual .raw files and SEQUEST .out files for a given analysis are analyzed together through the TPP to calculate the most accurate peptide probabilities, protein probabilities, and ratios for a given analysis.

3.2 Preparation of Datasets

3.2.1 Proteins in differentially expressed cisplatin-resistant vs. cisplatin-sensitive ovarian cancer cells

The protein quantification data was stored in Oracle schema Sysbio (see appendix 1). 574 differentially expressed proteins with q-value (false discovery rate) <=0.10; both up- and down-regulation values or 141 proteins (with q-value <=0.05) were generated by mass spectrometry based proteomics experiment. Proteins were mapped into IPI database IDs. These IPI identifiers were converted into UniProt IDs in order to integrate this data set with all other annotated public data. 119 of the 141 proteins (0.05 q-value threshold) were successfully mapped and converted (see appendix 2), using the International Protein Index (IPI) database[74] downloaded in February 2006, the UniProt database downloaded in November 2005[75], and additional internally curated public database mapping tables. Similarly, 451 out of the 574 proteins with the less strict threshold (q-value <=0.10) were mapped from IPI IDs to UniProt IDs.

3.2.2 Differentially expressed proteins identified from Grr1 knock-out yeast vs. wild-type yeast

For each protein identified in mass spectrometry experiment, there are two measures: (i) the probability that the identification is correct (output from ProteinProphet) and (ii) the relative abundance ratio and its standard error (output from ASAPratio).

Since some proteins might be identified by more than one experiment, one can improve the reliability and accuracy of the two measures by combining the estimates from each experiment. If a protein is identified by *k* experiments, labeled r_1, r_2,… r_k, then the summarized probability is calculated as:

$$P_{id} = 1 - \prod_{i=1}^{k} (1 - P_{r_i}) \ ,$$

Where P_{r_i} is the probability measure from experiment r_i.

To summarize the estimate of the relative abundance ratio, we use a linear combination of the estimate at the log10 scale from each experiment. The weight is determined so that the summarized estimate has the lowest standard error among all possible linear combinations. Then the z-score is calculated by dividing the summarized estimate by its standard error for each protein. The local false discovery rate approach proposed by Effron[76] is applied to the z-scores to calculate the probability that the relative abundance ratio is different from 1 (P_{ratio}). Finally, we take $P = P_{id} \times P_{ratio}$ as the final confidence measure that a protein is differentially expressed between the two samples. In other words, to be claimed as “differentially expressed”, a protein needs to have high confidence in its identification and high confidence in its differential abundance. 184 proteins were selected (Combined Probability>=0.8) from Grr1 knock-out vs. wild-type yeast mass spectrometry experiment (see appendix 3).

3.2.3 Protein interactome data

The primary source of human data comes from the Online Predicted Human Interaction Database (OPHID) [58], which were downloaded in February 2006. It contains more than 47,213 human protein interactions among 10,577 proteins identified by UniProt accession numbers. After mapping the proteins in OPHID to UniProt IDs, we recorded 46,556 unique protein interactions among 9,959 proteins. Note that even though more than half of OPHID entries are interacting protein pairs inferred from available lower organisms onto their human orthologous protein pair counterparts, the statistical significance of these predicted human interactions was confirmed by additional evidences according to OPHID and partially cross-validated according to our previous experience [77]. We assigned a heuristic interaction confidence score to each protein interaction, based on the type and source protein recorded in OPHID according to a method described in [77]. We call this data set PiD_0.

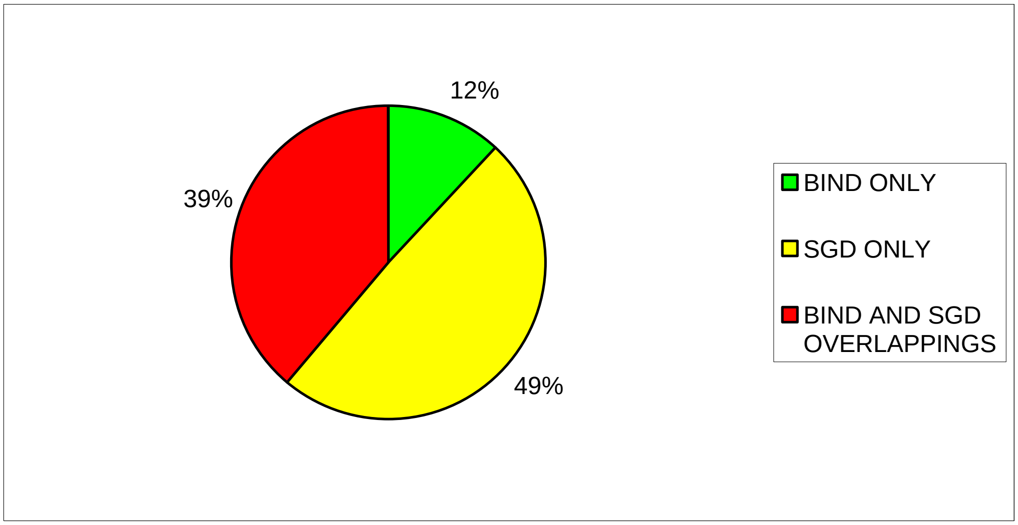


Figure 3.1 Yeast interactome data source from BIND and SGD. The percentages show the proportion of the non-redundant interacting pairs of each category among the combined non-redundant interacting pairs from BIND and SGD.

The source of yeast interactome data was the Saccharomyces Genome Database (SGD) [58], Biomolecular Interaction Network Database (BIND), and a small set of in-house manually curated data by our biology group (Goebl). The data from SGD and

BIND were downloaded in February 2006. Figure 3.1 summarized the percentage of overlappings of the interactome data from SGD and BIND. A total of 25,418 non-redundant interactions were obtained after combining the 3 interactome datasets and the intensive processing (see Figure 3.2 for the data processing flow chart). We call this interactome dataset **SBG**. Non-redundant interactions are defined as the interactions that only contain unique interacting pairs. The same interaction detected by different methods or published in different papers is counted as one unique pair. For each interacting pair in SBG, we calculated a reliability score based on the scoring model developed (see section 3.3).

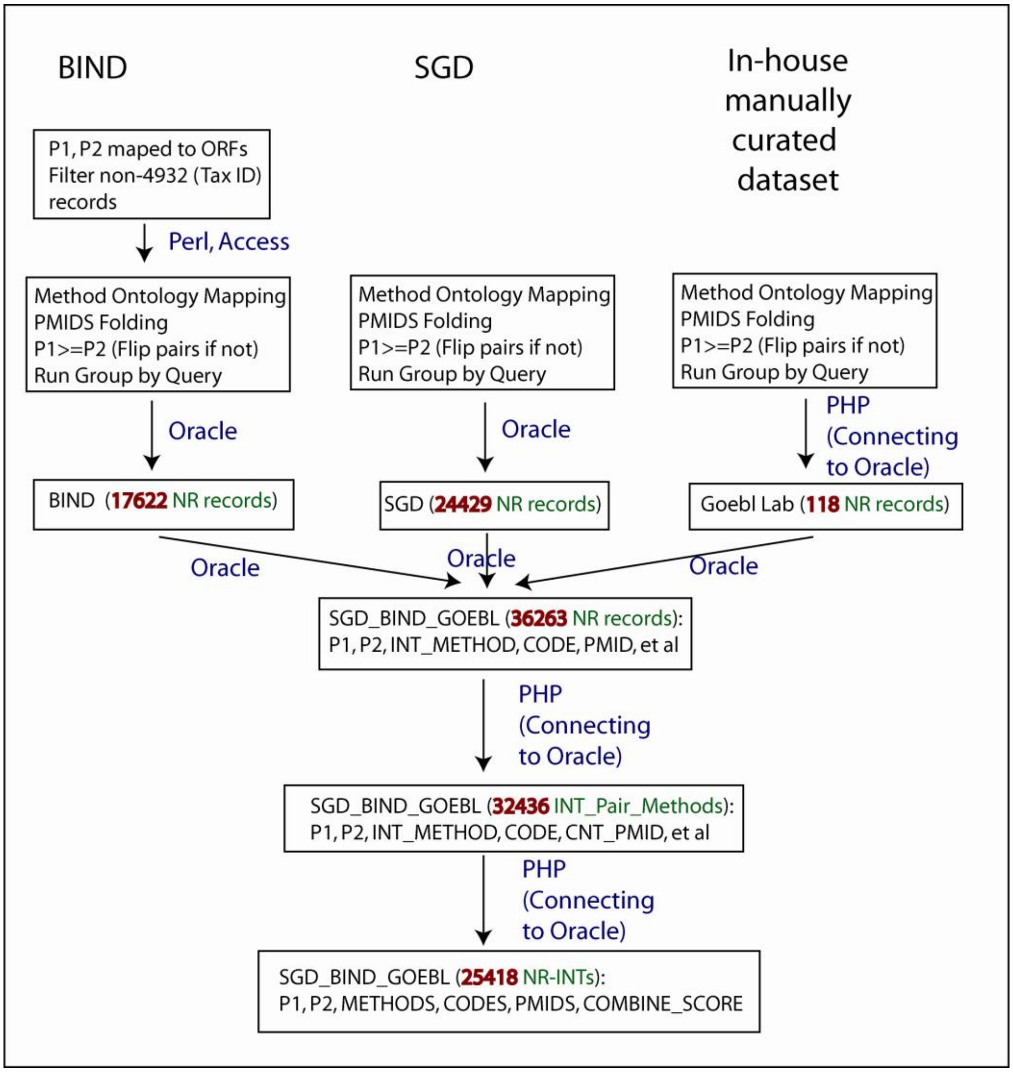

BIND
SGD
In-house manually curated dataset
P1, P2 maped to ORFs
Filter non-4932 (Tax ID) records
Perl, Access
Method Ontology Mapping
PMIDS Folding
P1>=P2 (Flip pairs if not)
Run Group by Query
Method Ontology Mapping
PMIDS Folding
P1>=P2 (Flip pairs if not)
Run Group by Query
Method Ontology Mapping
PMIDS Folding
P1>=P2 (Flip pairs if not)
Run Group by Query
Oracle
Oracle
PHP (Connecting to Oracle)
BIND (17622 NR records)
SGD (24429 NR records)
Goebl Lab (118 NR records)
Oracle
Oracle
Oracle
SGD_BIND_GOEBL (36263 NR records):
P1, P2, INT_METHOD, CODE, PMID, et al
PHP (Connecting to Oracle)
SGD_BIND_GOEBL (32436 INT_Pair_Methods):
P1, P2, INT_METHOD, CODE, CNT_PMID, et al
PHP (Connecting to Oracle)
SGD_BIND_GOEBL (25418 NR-INTs):
P1, P2, METHODS, CODES, PMIDS, COMBINE_SCORE

Figure 3.2 Interactome data integration flow chart

3.2.4 Noise-introduced human protein interactome data

To test how robust the final computational results would hold up against noise, which is commonly believed to exist in large portions of the public protein interaction data set, we generated two additional human protein interaction data sets, PiD-a20 and PiD-r20.

For PiD-a20, we add "protein interaction noise" by randomly connecting protein pairs from the set of 9,959 unique proteins for as many times as necessary to eventually generate 120% * 46,556= 55869 unique interactions. Therefore, we generate 20% new and unique "noisy" interactions in the PiD-a20 data set.

For PiD-r20, we eliminate "protein interaction noise" by randomly removing protein interaction pairs from the total 45,556 initial pairs of protein interactions in PiD_0 to eventually reduce the total number of protein interactions down to (1-20%) * 46,556= 37,243. Therefore, 80% of original interactions are kept intact in the PiD-r20 data set.

3.2.5 Gene annotation data

The human gene annotation database was downloaded from http://www.genmapp.org in January 2006. The whole annotation database (in MS Access) was then migrated to Oracle 10g. Human proteome GO annotation was performed based on human gene GO annotation and human gene ID to protein UniProt ID mappings.

The yeast gene annotation database was downloaded from www.genmapp.org in January 2006. This database (in MS Access) was migrated to Oracle 10g. We also downloaded additional annotation datasets from other websites such as http://www.yeastgenome.org in January and February. Based on these datasets, we designed and implemented our yeast gene annotation database (see Figure 3.3). Yeast proteome GO annotation was performed based on yeast gene GO annotation and yeast gene ID to ORFs mappings curated internally.

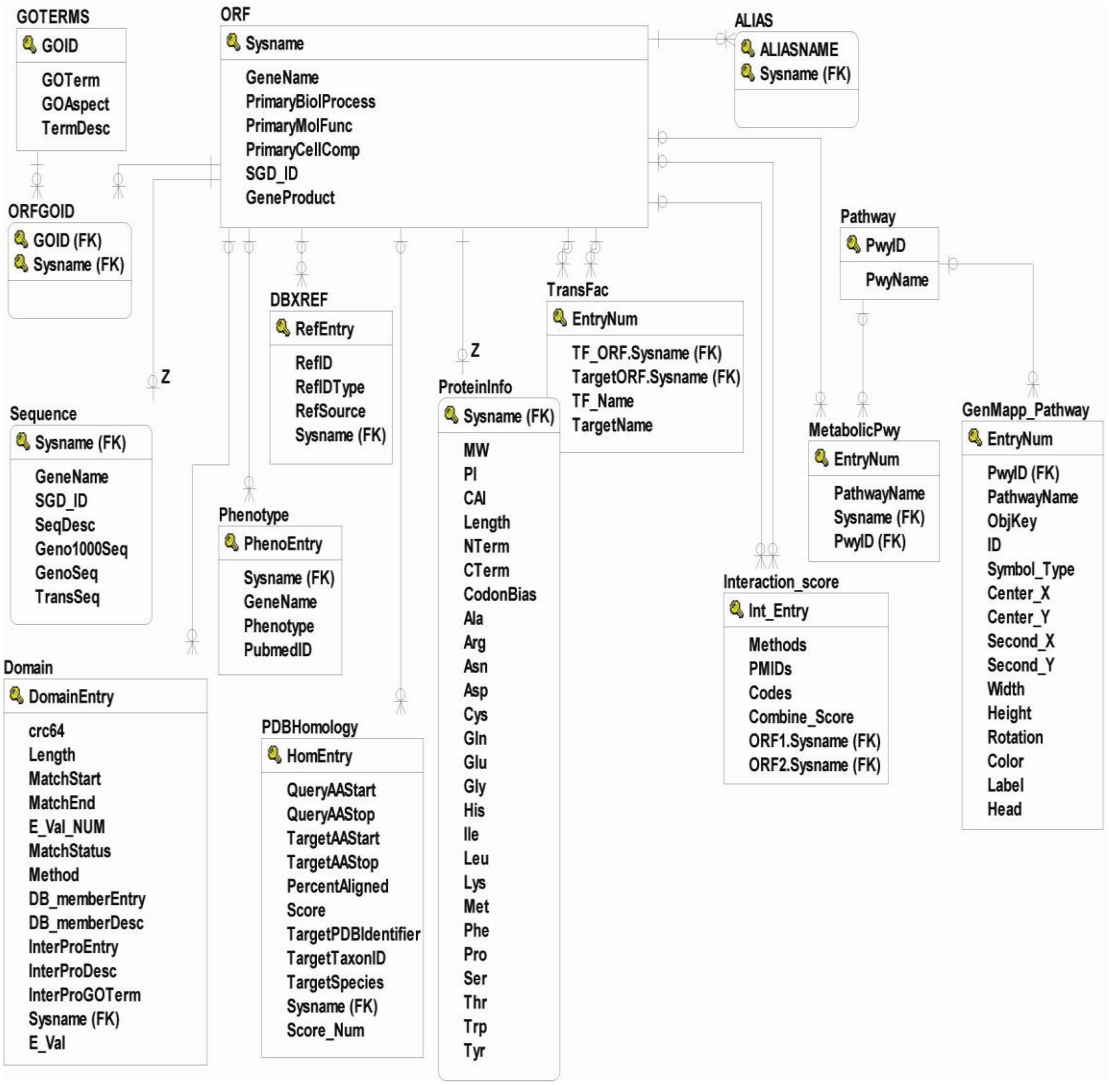


Figure 3.3 ERD diagram of yeast annotation database stored in Oracle 10g

3.2.6 Interacting protein categorical annotation data

Each GO term from the human or yeast protein annotation data was annotated with its minimal GO level number in the GO term hierarchy. Each GO term’s higher-level parent GO terms (multiple parent GO terms are possible) up to GO level 1 (three GO terms at this level: molecular function, cellular components, and biological processes) are also traced and recorded in an internally curated GO annotation table. When calculating interacting protein GO category information, we use this internally curated GO term table to map all the low-level GO term IDs (original GO Term ID) used to annotate each protein to all the GO term IDs' high-level GO term IDs (folded GO Term ID). For this study, we designate that all the folded GO term ID should be at GO term hierarchy Level = 3. Note that our method allows for multiple GO annotation Term IDs (original or folded) generated for each protein ID on purpose. Therefore, it is possible for a protein or a protein interaction pair to appear in more than one folded GO term category or more than one folded GO term interacting category pairs.

3.3 Protein-Protein Interaction Scoring Model

The reliability score of a pair of interaction can be assigned based on what experimental methods detected the interaction, how many different methods were used, and how many different papers have published the same interaction.

Our scoring model was developed in 3 steps:

First, we mapped the interaction methods stored in SGD (see table 3.1) or BIND (see table 3.2) into certain codes: for SGD, the code begins with "s"; for BIND, the code begins with "b". Then we created our metadata (see table 3.3) to unify the experimental methods for interacted pairs stored in SGD and BIND. We created the code for the unified method, which begins with "G". For each unified term, a reliability score was assigned based on the characteristics of the experimental method. Generally, interactions identified from low throughput experiments are more reliable than from high throughput experiments, for example, the method "Two Hybrid" was assigned the lowest score "0.1". Based on this, an interaction pair *j* identified by experimental method *i* can be assigned a base score of $S_{0ji.}$

(2) For interaction pair *j*, a specified experimental method *i* can associate with certain number ($C j_i$) of unique PubMed IDs. A maximum number of publications can be calculated among all methods for the same pair of interaction. The adjusted rate δji

for experimental method *i* of a certain interacted pair *j* can be calculated as (see figure 3.4):

$$\delta ji = \frac{Cji - 1}{\max(\max Cji - 1,\ 1)} \times 10\%$$

(Where j denotes the jth unique interaction pair, i denotes the ith experimental method)

The adjusted score for experimental method *i* of a specified interaction pair *j* can be calculated as (see figure 3.4):

$$S\, j_i = S_{0ji}\,(\,1+\ \delta\, ji)$$

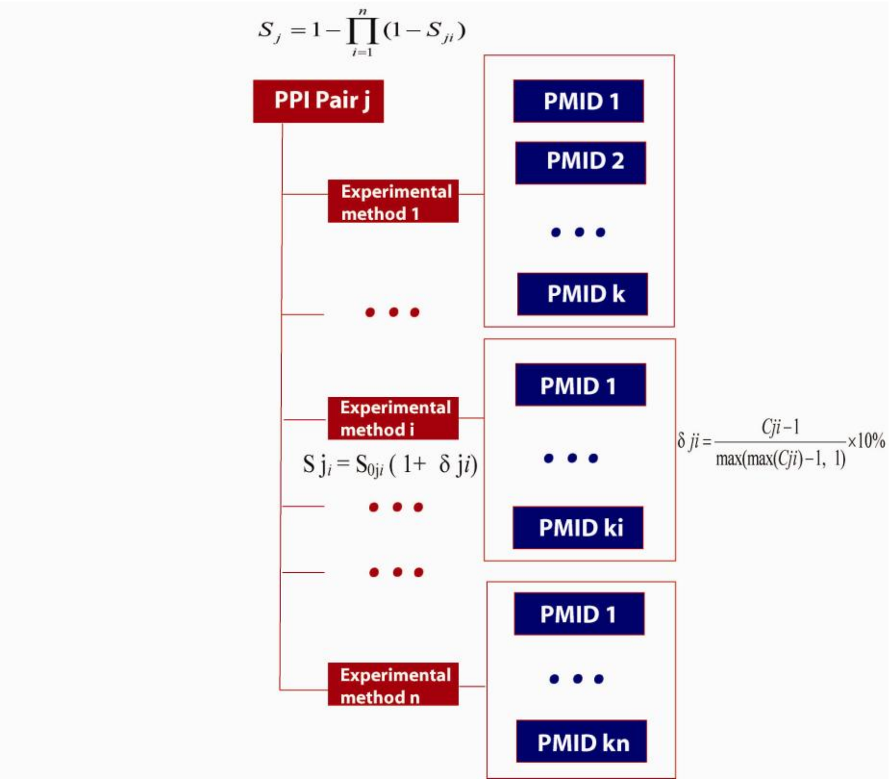


Figure 3.4 Protein interaction reliability score calculation algorithm and formulas. PMID stands for PubMed ID.

(3) Similar to [78], we combine Sj_1, Sj_2, …, Sj_i, …, Sj_n and calculate a final score Sj for the specified interaction pair *j* (see figure 3.4).

$$S_j = 1 - \prod_{i=1}^{n} (1 - S_{ji})$$

Table 3.1 Experimental methods for interacting pairs stored in SGD.

SGD Method Code	SGD Method
s1	Affinity Capture-MS
s2	Affinity Chromatography
s3	Affinity Precipitation
s4	Biochemical Assay
s5	Dosage Lethality
s6	e-map
s7	Purified Complex
s8	Reconstituted Complex
s9	Synthetic Growth Defect
s10	Synthetic Lethality
s11	Synthetic Rescue
s12	Two-hybrid

Table 3.2 Experimental methods for interacting pairs stored in BIND.

BIND Method Code	BIND Method
b1	(Blank)
b2	affinity-chromatography
b3	autoradiography
b4	colocalization
b5	competition-binding
b6	cross-linking
b7	deuterium-hydrogen-exchange
b8	electron-microscopy
b9	electron-spin-resonance
b10	elisa
b11	equilibrium-dialysis
b12	far-western
b13	fluorescence-anisotropy
b14	footprinting
b15	gel-filtration-chromatography
b16	gel-retardation-assays

b17	hybridization
b18	immunoblotting
b19	immunoprecipitation
b20	immunostaining
b21	interaction-adhesion-assay
b22	light-scattering
b23	mass-spectrometry
b24	membrane-filtration
b25	monoclonal-antibody-blockade
b26	not-specified
b27	other
b28	phage-display
b29	resonance-energy-transfer
b30	sucrose-gradient-sedimentation
b31	surface-plasmon-resonance-chip
b32	three-dimensional-structure
b33	transient-coexpression
b34	two-hybrid-test

Table 3.3 Metadata for experimental methods

SGD and BIND Method Code	Code	Unified Term	Reliability Score
b2, s1, s2, s3, b19	G1	Affinity_Purification	0.8
b6	G2	Cross_Linking	0.5
b10	G3	Elisa	0.7
b28	G4	Phage_Display	0.1
b29	G5	Resonance_Energy_Transfer	0.4
b34, s12	G6	Two_Hybrid	0.1
s6	G7	E_Map	0.8
s9, s10	G8	Synthetic_Growth_Defect	0.8
s11	G9	Synthetic_Rescue	0.8
s7	G10	Purified_Complex	0.8
s5	G11	Dosage_Lethality	0.8
s4	G12	Biochemical_Assay	0.8
s8	G13	Reconstituted_Complex	0.6

b3, b4 ~ b9	G99	Other	0.1
b11, b12 ~ b18	G99	Other	0.1
b20, b21 ~ b27	G99	Other	0.1
b1, b30 ~ b33	G99	Other	0.1

3.4 Network Expansion

We derive differentially expressed protein interaction sub-network using a nearest-neighbor expansion method described in [77]. We call the original list of differentially expressed proteins (119 proteins in ovarian cancer study or 184 proteins in yeast Grr1 knock-out study) seed (S) proteins and all the protein interactions within the seed interactions (or S-S type interactions). After expansion, we call the collection of seed proteins and expanded non-seed (N) proteins sub-network proteins (including both S and N proteins); we call the collection of seed interactions and expanded seed-to-non-seed interactions (or S-N type interactions) sub-network protein interactions (including both S-S type and S-N type interactions). Note that we do not include non-seed-to-non-seed protein interactions (or "N-N" type interactions) in our definition of the sub-network, primarily because the N-N type of protein interactions often outnumbered total S-S and S-N types of protein interaction by several folds with molecular network context often not tightly related to the initial seed proteins and seed interactions. The only occasion to consider the N-N type interactions is when we calculate sub-network properties such as node degrees for proteins in the sub-network.

3.5 Network Visualization

We use Spotfire DecisionSite Browser 7.2 to implement the 2-dimensional functional categorical crosstalk matrix for human ovarian cancer drug resistance study. To perform interaction network visualization, we used ProteoLens[79]. ProteoLens has native built-in support for relational database access and manipulations. It allows expert users to browse database schemas and tables, query relational data using SQL, and customize data fields to be visualized as graphical annotations in the visualized network.

3.6 Network Statistical Examination

Since the seed proteins are those that are found to display different abundance level between two different cell lines via mass spectrometry, one would expect that the network "induced" by them to be more "connected" in the sense that they are to a certain extent related to the same biological process(es). To gauge network "connectivity", we introduced several basic concepts. We define a **path** between two proteins A and B as a set of proteins P1, P2,…, Pn such that A interacts with P1, P1 interacts with P2, …, and Pn interacts with B. Note that if A directly interacts with B, then the path is the empty set. We define the **largest connected component** of a network, as the largest subset of proteins such that there is at least one path between any pair of proteins in the network. We define the **index of aggregation** of a network as the ratio of the size of the largest connected component of the network to the size of the network by protein counts. Therefore, the higher the index of aggregation, the more "connected" the network should be. Lastly, we define the **index of expansion** of a sub-network as the ratio of S-S type interactions among seed proteins over all seed and expanded sub-network interactions (S-S and S-N types). The higher the index of expansion, the more relevant roles seed proteins plays in the network.

To examine the statistical significance of observed index of aggregation and index of expansion in expanded protein networks, we measure the likelihood of the topology of the observed sub-network under random selection of seed proteins. This is done by randomly selecting 119 proteins (in ovarian cancer study) or 184 proteins (in yeast Grr1 knock-out study), identifying the sub-network induced/expanded, and calculating sub-network indexes accordingly. The same procedure is repeated n=1000 times to generate the distribution of the indexes under random sampling, with which the observed values are compared to obtain significance levels (for details, refer to [77]).

3.7 Significance of Testing of GO Categories and GO-GO Categories

To assess how significantly the seed proteins (119 in human study and 184 in yeast study) in the subnetwork are distributed across their specific GO function categories, we hypothesize the task as observing the outcome of a random draw of the same number of proteins from the pool of proteins in the whole interacting network (9959 proteins in human study and 5240 in yeast study). Then the count in a certain GO category follows a hypergeometric distribution. A p-value is calculated based on the hypergeometric distribution to evaluate the likelihood that we observe an outcome under random selection of a subset of proteins (119 in human and 184 in yeast) that is at least as "extreme" as what we have observed. Note "extreme" either implies an unusually large (over-representation) or usually small (under- representation) number. Let x be the count of the seed proteins that falls in a function category in the subnetwork, n is the sample size (119 in human study, or 184 in yeast study), N is the population size (9,959 in human study, or 5240 in yeast study), and k=corresponding count in OPHID, then the p-value for over/under-representation of the observed count can be calculated as:

Over representation:

$$p = \Pr[X \geq x \mid n, N, k] = \sum_{i=x}^{\min(n,k)} \binom{k}{i} \binom{N-k}{n-i} / \binom{N}{n}$$

Under representation:

$$p = \Pr[X \leq x \mid n, N, k] = \sum_{i=0}^{x} \binom{k}{i} \binom{N-k}{n-i} / \binom{N}{n}$$

We also expand the protein list from the 184 seed proteins to 1251 sub-network proteins in yeast study, and calculate a p-value for randomly drawing of 1251 proteins from the pool of 5240 proteins in SBG based on its hypergeometric distribution.

Similarly, we can use the above formula to assess how significantly the protein interactions from the seeded subnetwork are distributed across specific GO-GO functional interaction categories. For a GO-GO functional interaction category, we refer to a pair of GO categories, which are derived by aggregating all the protein-protein interaction pairs with the same pairing of GO annotation categories for the interacting proteins. For example, if 3 protein interactions share annotation category A in one side of the interaction, and annotation category B in the other side of the interaction, we say that A-B is a functional interaction category with an observed count of 3. To identify significant GO-GO functional interaction category in the seeded subnetwork, we hypothesize the task as the observing the outcome of a random draw of 1,723 pairs from the pool of 46,556 pairs in OPHID in human study (or random draw of .1,698 pairs from the pool of 25,418 pairs in SBG in yeast study). Then the count of a certain GO-GO function interaction category follows a hypergeometric distribution. A p-value for over/under-representation of the observed count can be calculated similarly, based on the hypergeometric distribution. Since tests of over/under representation of various categories are correlated with one another (over representation of one category could imply under representation of other categories), we also control the false discovery rate (FDR) using method developed by Benjamini and Yekutieli.[80]

3.8 Validation of Under-Represented GO Categories

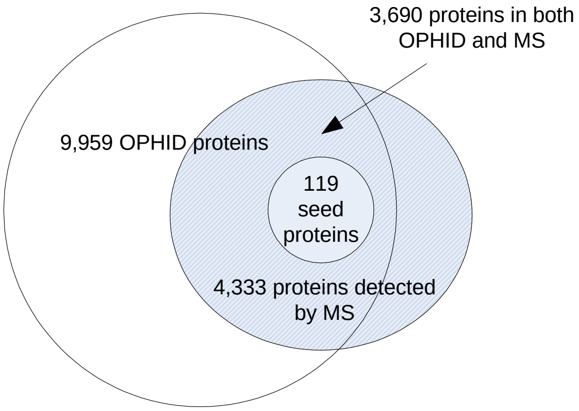

3,690 proteins in both OPHID and MS
9,959 OPHID proteins
119 seed proteins
4,333 proteins detected by MS

Figure 3.5 Illustration showing the overlap of 9959 OPHID proteins and 4333 proteins detected by MS experiments. 119 seed proteins is a high-confidence subset of the overlapped proteins.

Proteomics techniques are generally known for problems with false positives and false negatives, primarily for reasons such as complex digested peptide samples, noisy un-separated peptide peaks, and computationally intensive protein/peptide identifications that cannot afford to take all post-translational modifications into account. Since we control false positives by choosing 119 high-confidence seed proteins in this study, false negatives, instead of false positives, are a potential concern. Therefore, when we interpret over-/under- representation of proteins in GO functional categories or GO-GO functional interaction categories, over-representation results are likely under-exaggerated and will remain true, but under-representation results are likely over-exaggerated and needs additional validation or some adjustment.

We take 4,333 of all reported proteins, which includes proteins identified with both high and low confidence, from the MS search software, and overlap this set with all the 9,959 proteins found in OPHID. Out of 4,333 raw UniProt IDs, 3,690 of which can be further mapped to OPHID interaction human database. The 3,690 is then assumed to be the upper limit of instrument/software detectable proteins. When re-examining over-/under- representation GO functional categories, we let n=3,690, N=9,959, k=corresponding count in OPHID, and use the same formula introduced in section 2.6 to calculate significant protein over/under- representation. This relationship is illustrated in Figure 3.5.

3.9 Drill-Down of Significant Categories

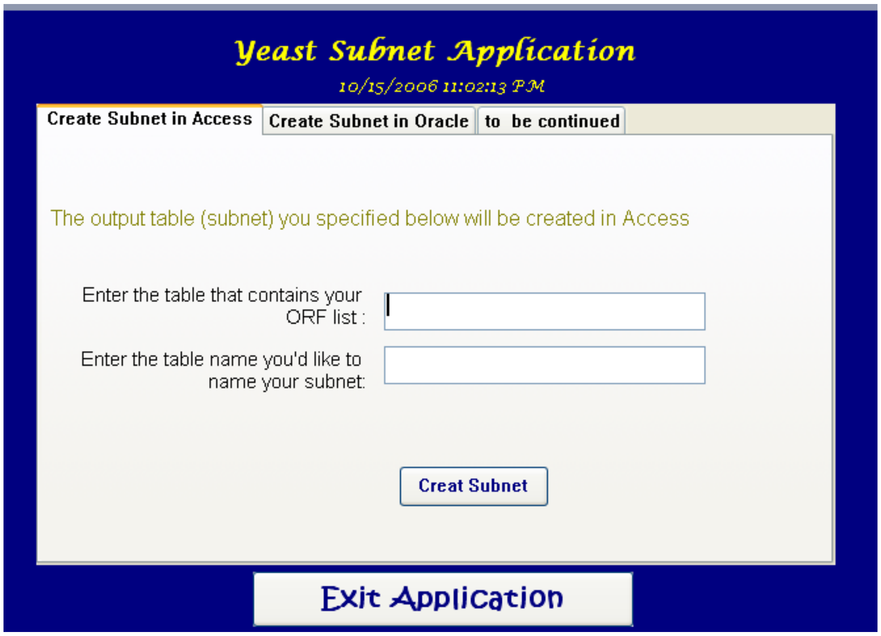
Once certain GO functional categories or GO-GO functional interaction categories are determined to be significant, they become candidates for subsequent “**drill-down**” examinations. For drill-down of GO functional categories, we refer to exploration of the next-level GO functional annotation by tracing down the GO structure and re-calculating the significance value, based on each protein’s new next-level GO functional annotation labels, using methods described in section 2.6. For drill-down of GO-GO functional categories, we refer to exploring the next-level GO-GO functional annotations by tracing both proteins of the interaction pair down the GO structure and re-calculating the significance value. The new next-level GO-GO functional annotation categories consist of all paired combinations of sub GO functional categories. The use of drill-down allows us to zoom in our attention to detailed biologically interesting categories to obtain further insights in enriched molecular functions and biological processes without incurring a huge computational cost at the very beginning of the exploration.

3.10 Scoring of Significant Proteins in the Sub-Network

Protein ranking analysis was performed in MS Access front-end database which connects to the Oracle back-end database. First, 184 differentially expressed proteins were imported (with replacement) into linked Oracle table from the application interface (see Figure 3.6) after correct login information was verified, then the application automatically create the sub-network data by querying the linked Oracle SBG interactome dataset. We calculated ranking scores for the significant proteins in the sub-network using the heuristic relevance scoring formula[81]:

$$R_i = 2 * LOG(\sum_{j=1}^{k_i} S_{ij}) - LOG(k_i)$$

Where R*i* is the ith seed protein ranking score, K*i* denotes its connectivity, and S*ij* denotes its interaction reliability score with the jth partner.



Yeast Subnet Application

10/15/2006 11:02:13 PM

The output table (subnet) you specified below will be created in Access

Enter the table that contains your ORF list :

Enter the table name you'd like to name your subnet:

Figure 3.6 Application for the yeast subnet construction

Our ranking analysis was built on the hypothesis: the significance of a protein's contribution in the network depends on its ability to connect to other proteins in the network and the reliability of the detected interactions. The higher the connectivity and reliability, the higher the ranking score should be.

4. RESULTS

Case Study 1. Ovarian Cancer Drug Resistance Case Study

This part was written based on the published result[2], where I am one of the primary contributing members. The use of the material was granted with the permission from participating contributors.

4.1 Activated Protein Interaction Sub-Network Properties

The network topology for the protein interaction sub-network expanded from seed proteins was examined. The resulting protein interaction sub-network (core sub-network) consists of 1,230 seed and non-seed proteins in 1,723 sub-network interactions (including 17 S-S type interactions and 1,706 S-N type protein interactions). The node degree frequency distributions were plotted in Figure 4.1, where the whole human protein interaction network from OPHID (labeled "network") is also shown. As expected, both the network and the sub-network (full) display good "scale-free" property. These results also show that the cisplatin resistant activated sub-network (full) contains more "hubs" than "peripheral" proteins to form a cohesive functional sub-network. The core sub-network, while perhaps limited in size, shows "scale-free like" distribution, although hubs in the sub-network (core) are more distinctively identifiable than overly abundant peripheral nodes by high node degree counts.

Other network features for the core sub-network are also examined. The largest connected component (defined in the Method section; ibid) of the sub-network consists of 1193 proteins. The index of aggregation is 1193/1230=97.0%. The index of expansion as the percentage of S-S type interactions (17) over the core sub-network interactions (1723), i.e., 17/1723=0.96%. The index of aggregation has a p-value of less than 0.001 (upper tail) and the index of expansion is 0.06 (upper tail) A significant and high network index of aggregation suggests that the core sub-network has connectivity structures that are not random by nature. This correlates well with the node degree distribution in Figure 4.1, where an exceptionally large number of hubs are shown to exist.

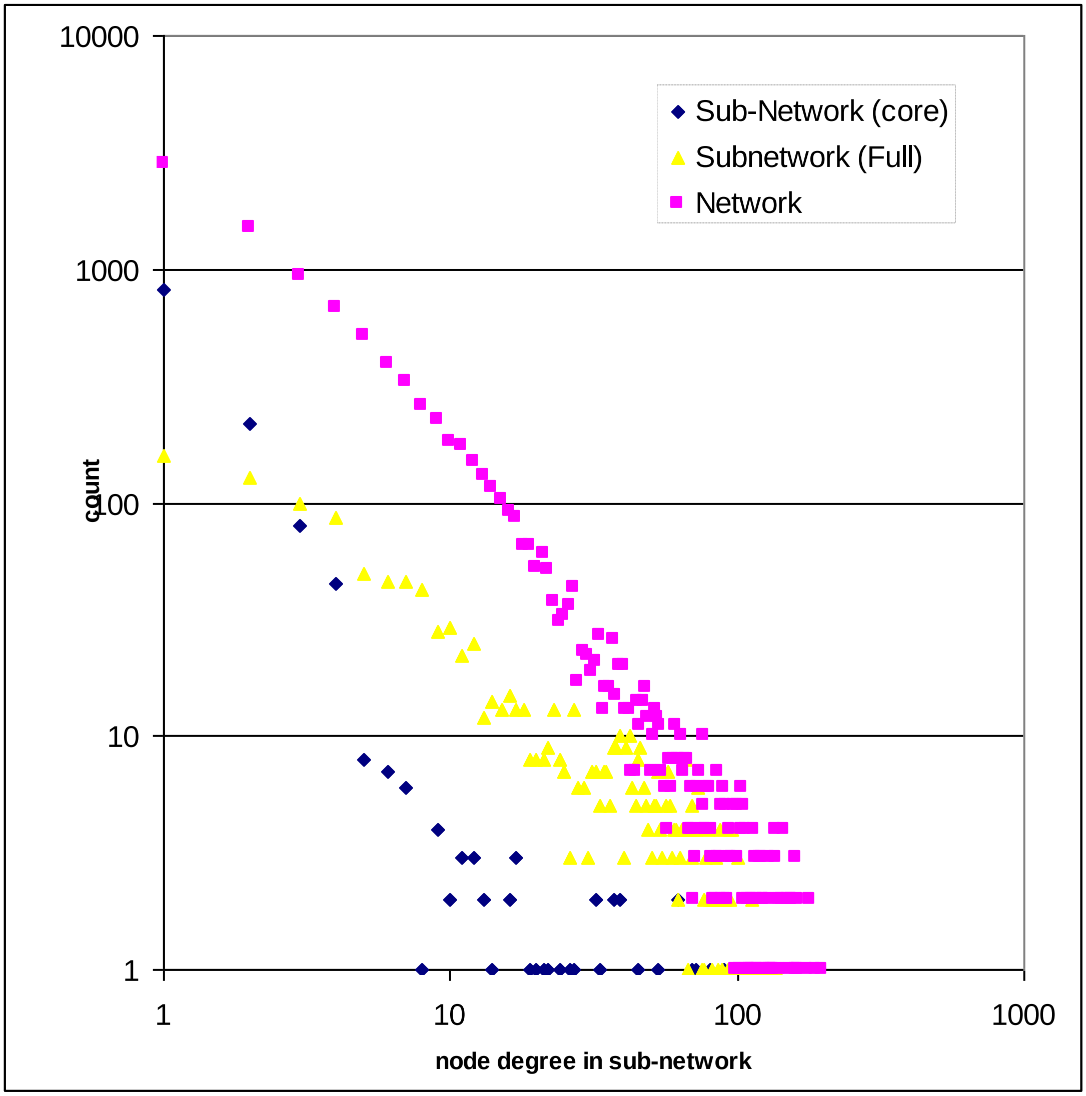


Figure 4.1 Node degree distribution of the sub-networks (core or full) in comparison with the human protein interaction network.

4.2 Analysis of Activated Protein Functional Category Distributions

Although GO-based functional category analysis can be done routinely using many existing bioinformatics methods [3], the inclusion of protein interaction network context has not been previously described. In this analysis, we are more interested in enriched protein categories in the cisplatin-response functional process. This includes both up-regulated and down-regulated proteins. Therefore, we transformed protein-protein interaction sub-network to GO cross-talk sub-network. Enriched protein functional categories were discovered among differentially expressed seed proteins and its immediate protein interaction sub-network nearest interaction partners.

Table 4.1 shows significantly enriched GO categories in the sub-network. 17 GO categories were filtered from 70 GO categories (data not shown). The filter criteria are 1) the P-value over- or under- representation must be within 0.05 and 2) the total category count of GO in the whole network is greater than 10. In **GO_TERM** column, we have listed three types of information: level 3 GO terms, GO term category type ('C' for cellular component, 'F' for molecular function, and 'P' for biological process; in parenthesis preceding the dash), and GO identifier (seven digit number following the dash in parenthesis). In the **ENRICHMENT** column, we listed two types of counts of proteins with GO annotation levels falling in the corresponding category: within core sub-network and whole network (in parenthesis). In the **PVALUE** column, we have listed two numbers: the p-value from the significance test of whether there is an over- or an under- representation (two numbers separated by a '/') of an observed GO term category count in the sub-network. In the last **CONCLUSION** column, '++' suggests significant over-representation when the false discovery rate (FDR) is controlled at 0.05, '--' suggests significant under-representation when FDR controlled at 0.05, '+' to suggest insignificant over-representation when FDR controlled at 0.05 but significant overrepresentation at native p-value=0.05, '-' to suggest insignificant over-representation when FDR controlled at 0.05 but significant overrepresentation at native p-value=0.05.

Table 4.1 Enriched GO categories in the sub-network Context. An asterisk indicates adjustment may be needed for further interpretation.

		PVALULE	
GO_TERM	**ENRICHMENT**	**(OVER/UNDER)**	**CONCLUSION**
membrane (C-0016020)*	7 (2034)	1/0	--
proton-transporting two-sector ATPase complex (C-0016469)	2 (12)	.009/1	+
non-membrane-bound organelle (C-0043228)	19 (834)	.005/.9980	+
organelle lumen (C-0043233)	2 (13)	.0101/.9996	+
proton-transporting ATP synthase complex (C-0045259)	2 (17)	.0171/.9990	+
proton-transporting ATP synthase complex\, catalytic core (C-0045261)	1 (1)	0.012/1	+
proton-transporting ATP synthase, catalytic core (C-0045267)	1 (1)	0.012/1	+
protein binding (F-0005515)	26 (1387)	.012/.9937	+
drug binding (F-0008144)	2 (12)	0.009/1	+
isomerase activity (F-0016853)	7 (69)	0/1	++
nucleotide binding (F-0000166)	23 (1205)	.0148/.9923	+
receptor activity (F-0004872)*	2 (642)	.9968/.0149	-
receptor binding (F-0005102)*	1 (422)	.9944/.0354	-
oxidoreductase activity (F-0016491)	12 (271)	0/1	++
Kinase regulator activity (F-0019207)	3 (50)	0.022/0.9970	+
metabolism (P-0008152)	67 (4634)	0.020/0.9875	+
response to biotic stimulus (P-0009607)*	1 (711)	1/0.0014	-
regulation of physiological process (P-0050791)*	17 (2129)	.9817/.0328	-
regulation of cellular process (P-0050794)*	15 (2182)	.9968/.0066	-

We also tested how robust the network is by introducing noise in protein interaction data sets. Two experiments were performed (for a description of the methods, see section 3.1.1) : "add20", in which we added 20% new randomly-selected connections between OPHID proteins to create a new OPHID proteins data set *PiD-a20*, and "remove20", in which we removed 20% existing randomly-selected connections between OPHID proteins to create a new OPHID proteins data set *PiD-r20*. Surprisingly, although the individual category counts fluctuate, all conclusions made through the above-described threshold values of p-value and FDR remain the same (this conclusion also remains true for high-level GO-GO category enrichment experiments in next section; results not shown). This suggests the significance of our discovery is robust against reasonable noise inherent in the protein interaction databases.

After the above analysis, we then re-examined all "under-represented categories" under a new false-negative controlled experiment to see if these under-representations have been "exaggerated" due to bias of the MS experimental methods. Therefore, we set up an experiment to observe the inherent bias (either over- or under- representation) in all detectable MS proteins overlapped with OPHID data sets (also described in section 3.1.6).

Table 4.2 Re-examination of under-represented seed protein functional categories

GO_TERM	P-VALUE, OVER-REPRESENTED (seed/background)	P-VALUE, UNDER-REPRESENTED (seed/background)	CONCLU-SION
Membrane (C-0016020)	1.0000 (.00000)	.00001 (1.0000)	--
receptor activity (F-0004872)	.99681 (.00002)	.01489 (.99998)	--
receptor binding (F-0005102)	.99439 (.99937)	.03550 (.00092)	?
response to biotic stimulus (P-0009607)	.99986 (1.0000)	.00144 (.00000)	?
regulation of physiological process (P-0050791)	.98169 (.00000)	.03276 (1.0000)	--
regulation of cellular process (P-0050794)	.99685 (.00000)	.00664 (1.0000)	--

Table 4.2 lists the results. Here *seed* experiment refers to the earlier experiment which we examined the enrichment (in this case, all under-representations) of 119 seed proteins; *background* experiment refers to the re-examination experiment which we examined the enrichment bias of 3690 MS detectable proteins also found in OPHID data set. When we observe significant over-representation of certain GO functional categories in the background, we make the conclusion that the category is indeed under-represented in the seed (marked as "--").When we observe significant under-representation of certain GO functional categories in the background, we make the conclusion that the category is not necessarily under-represented (or likely over-represented) in the seed (marked as "?" for inclusive).

From the above comprehensive analysis, we can obtain the following biological insights. First, proton-transporting ATP synthase activity is related to the cell cisplatin resistance function (see table 5 for the enriched GO categories), which may imply higher oxidative energy production capability among cancerous functions in cisplatin resistant cell lines over cisplatin sensitive cell lines. This is consistent with the existing findings: mitochondria -- "ATP factory", was considered to be a major target of cisplatin, leading to mitochondrial loss of energy production[82]. Second, although the protein interaction network in general is inherently enriched with proteins with "protein binding" capabilities (note 1412 proteins in the category from the whole network), the cisplatin-resistant cell line demonstrated an unusually high level of protein-binding activities; in addition, a broad spectrum of across-the-board drug-binding and nucleotide-binding mechanisms are all activated to fight again cisplatin-induced DNA damage in cancer cells. This suggests that many intracellular signaling cascades are intensely mobilized with cisplatin-resistance. Third, the data suggest that the location of the biological activities of cisplatin resistant response take place in cytoplasm or nucleus, rather than on "membrane". This correlates well with the previous hypothesis that transporters that are responsible for assisting with cisplatin import into the cell seem to become blocked in drug-resistant cells. This analysis gives essential clues to the overall picture of molecular signaling events for cisplatin resistant cell lines. We also obtained categorical enrichment data at lower GO levels than are shown in this section, using the drill-down method (for method, refer to section 3.1.7; results not shown), to obtain detailed views of biological process, molecular function, and cellular components.

4.3 Functional Category Cross-Talks

We developed a two-dimensional visualization matrix (extended from our technique described in [83]) to show significant cross-talk between GO categories in Figure 4.2 (only biological processes at level=3 are shown due to space constraints).

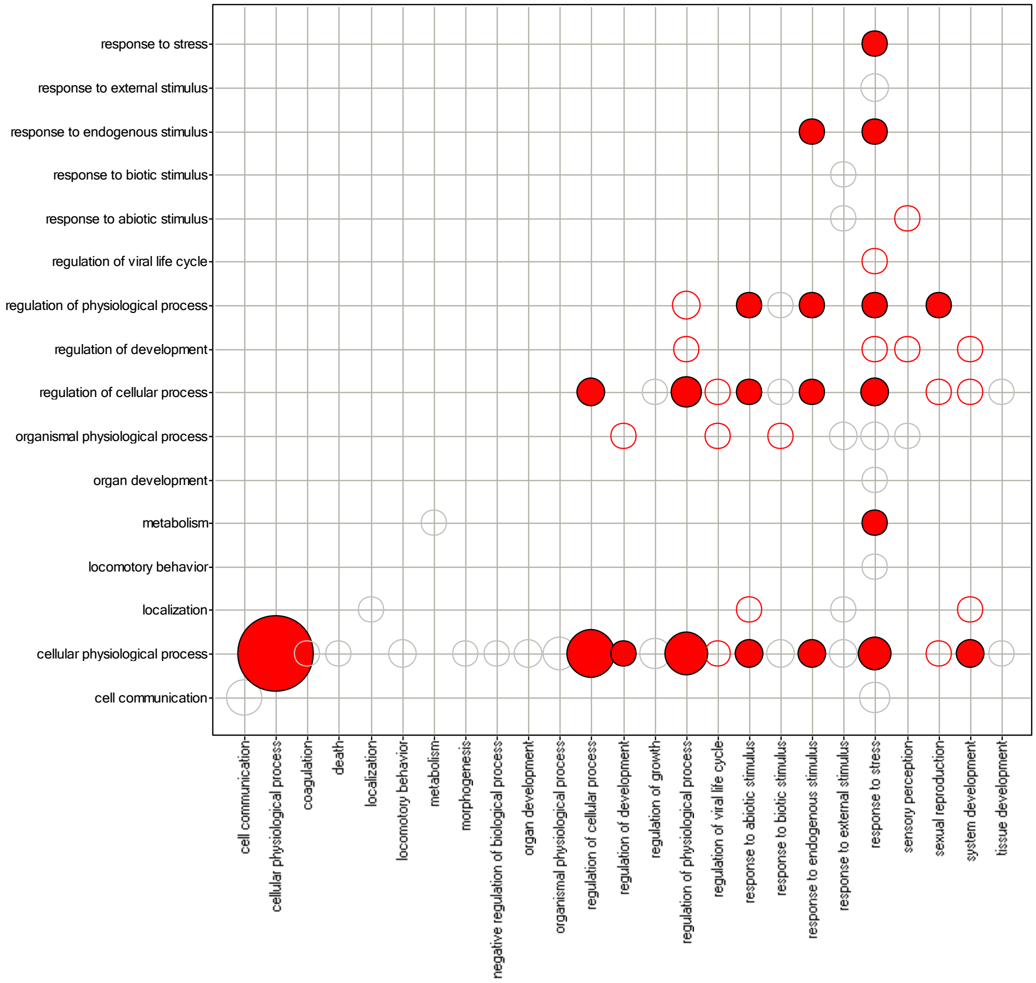


Figure 4.2 Significantly over-represented GO-GO interaction categories in seeded subnetwork. (only biological processes at level=3 are shown due to space constraints).

Table 4.3 Drill down of significant GO-GO functional category cross-talk. “Cellular Physiological Process” vs. “Cellular Physiological Process” at GO term level 4. Note only p-value <0.05 for over-representation are shown (FDR<0.05 cases are also in bold)

GO TERM #1	GO TERM #2	ENRICHMENT	P-VALUE
cell homeostasis (P-0019725)	transport (P-0006810)	18 (120)	**0**
transport (P-0006810)	transport (P-0006810)	75 (1377)	**0.0006**
regulation of cellular physiological process (P-0051244)	transport (P-0006810)	130 (2045)	**0**
cell cycle (P-0007049)	transport (P-0006810)	68 (708)	**0**
cell death (P-0008219)	transport (P-0006810)	45 (694)	**0.0002**
cell proliferation (P-0008283)	transport (P-0006810)	22 (238)	**0.0001**
cellular metabolism (P-0044237)	transport (P-0006810)	359 (6041)	**0**
cellular metabolism (P-0044237)	cell cycle (P-0007049)	253 (4439)	**0**
cellular metabolism (P-0044237)	cell proliferation (P-0008283)	85 (1412)	**0**
cell homeostasis (P-0019725)	cell organization and biogenesis (P-0016043)	11 (73)	**0.0001**
cellular metabolism (P-0044237)	cell homeostasis (P-0019725)	25 (279)	**0**
cellular metabolism (P-0044237)	cellular metabolism (P-0044237)	764 (17604)	**0**
cell homeostasis (P-0019725)	cell cycle (P-0007049)	5 (43)	0.0207
regulation of cellular physiological process (P-0051244)	cell cycle (P-0007049)	130 (2655)	0.0007
cell cycle (P-0007049)	cell cycle (P-0007049)	39 (702)	0.0084
cell organization and biogenesis (P-0016043)	cell cycle (P-0007049)	53 (936)	0.0017
cell homeostasis (P-0019725)	chromosome segregation (P-0007059)	1 (1)	0.037
regulation of cellular physiological process (P-0051244)	cell homeostasis (P-0019725)	13 (149)	0.0037

The size of each node is inversely proportional to the p-value of interacting categories. The color legends are: red (dark) for interacting categories that are significant when FDR controlled at 0.05; and gray (light) for interacting categories that are not significant when FDR controlled at 0.05. The figure 4.2 reveals additional interesting findings. First, cellular physiological processes are significantly activated in drug-resistant cell lines (the largest and reddest dot, at the bottom left corner). This could lead to further drill-down of protein interaction in the interacting category for biological validations (see Table 4.3 for an example). Second, these cellular physiological processes seem to be quite selective rather than comprehensive. For example, when looking at significant regulation of cellular response categories, significant cross-talk functional patterns strongly suggest the cellular and physiological responses arise from endogenous, abiotic, and stress-related signals (internalized cisplatin causing DNA damage and inducing cell stress). Using a cross-talk matrix such as this, cancer biologists can quickly filter out other insignificant secondary responses (such as cell growth, cell development shown) to establish a new prioritized hypothesis to test.

4.4 Visualization of the Activated Interaction Functional Sub-Network

In Figure 4.3, we show a visualization of the activated biological process functional network, using a recently developed software tool “ProteoLens”[79]. ProteoLens is a biological network data mining and annotation platform, which supports standard GML files and relational data in the Oracle Database Management System (for additional details, visit http://bio.informatics.iupui.edu/proteolens/). In the figure 4.3, in contrast with regular protein interaction network, we encode nodes as significantly over-/under- represented protein functional categories, and edges as significantly interacting protein functional categories. Several additional information types are also represented. The original abundance (by count) of each functional category is encoded in the node size. The p-values of activated **protein category significance** in the sub-network is encode as node color intensity, on a scale from light yellow (less significant) to dark red (more significant).

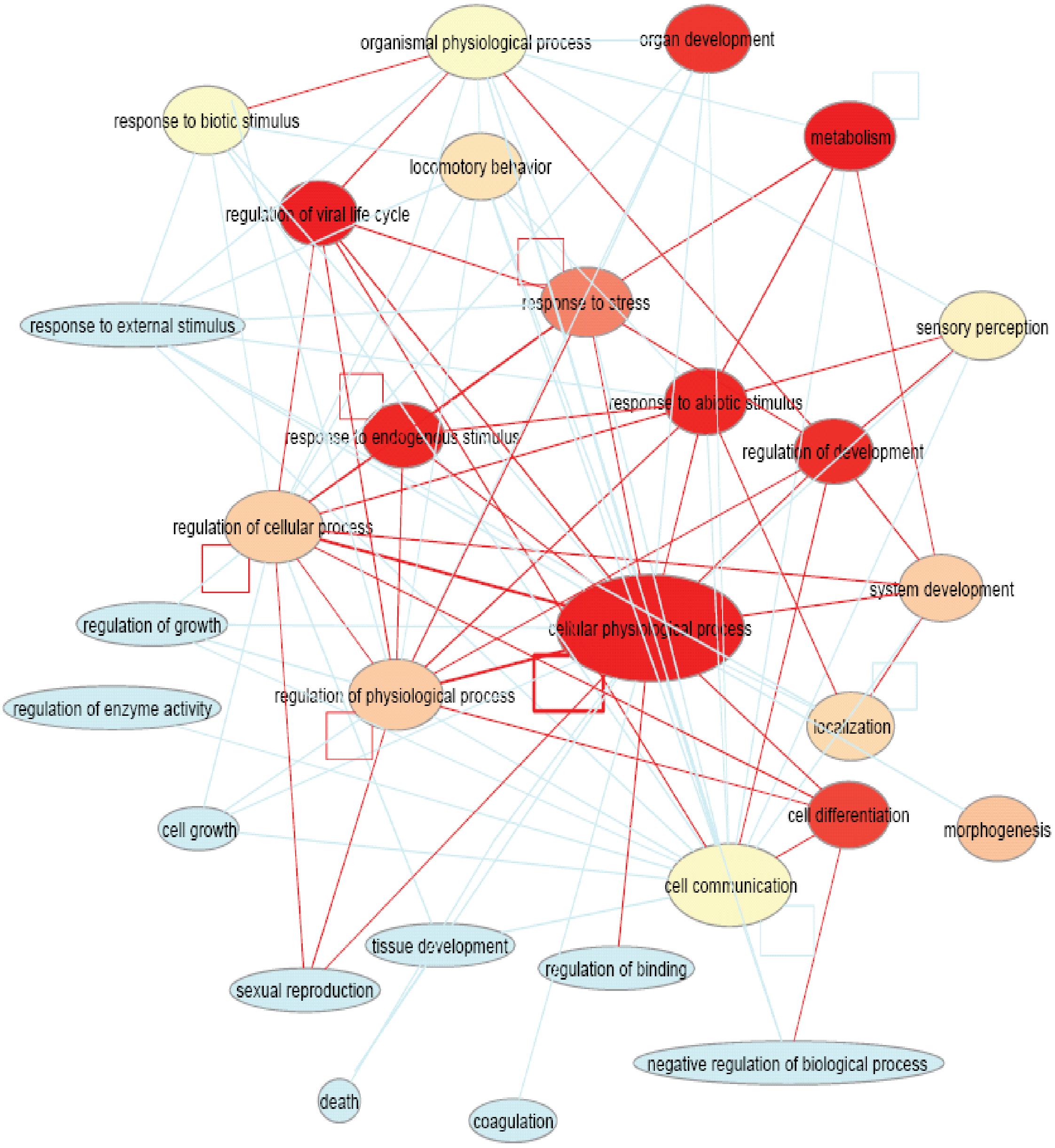

organismal physiological process
organ development
response to biotic stimulus
metabolism
locomotory behavior
regulation of viral life cycle
response to stress
sensory perception
response to external stimulus
response to abiotic stimulus
response to endogenous stimulus
regulation of development
regulation of cellular process
system development
regulation of growth
cellular physiological process
regulation of enzyme activity
regulation of physiological process
localization
cell growth
cell differentiation
morphogenesis
cell communication
tissue development
regulation of binding
sexual reproduction
negative regulation of biological process
death
coagulation

Figure 4.3 Activated biological process network in cisplatin-resistant ovarian cancer cells. Red-colored lines stand for "significant", while blue-colored lines stand for "not significant" (FDR=0.05)

From this figure 4.3 we can see that cisplatin-resistant ovarian cancer cells demonstrated significant cellular physiological changes, which are related to cancer cell's native response to stimulus that is endogenous, abiotic, and stress-related. Interestingly, we also observed that the regulation of viral life cycle also plays very significant roles in the entire drug resistant process. This previously unknown observation may be further examined at protein levels to formulate hypothesis about acquired cisplatin resistance in ovarian cancer.

Case Study 2. Yeast Grr1 Knock-Out Case Study

Case study 2 is the collaborative work among the biology group (Dr. Goebl and Josh), biostatistics group (Dr. Shen), and Informatics group (Dr. Chen and I). The manuscript is in preparation.

4.5 Activated Protein Interaction Sub-Network Properties

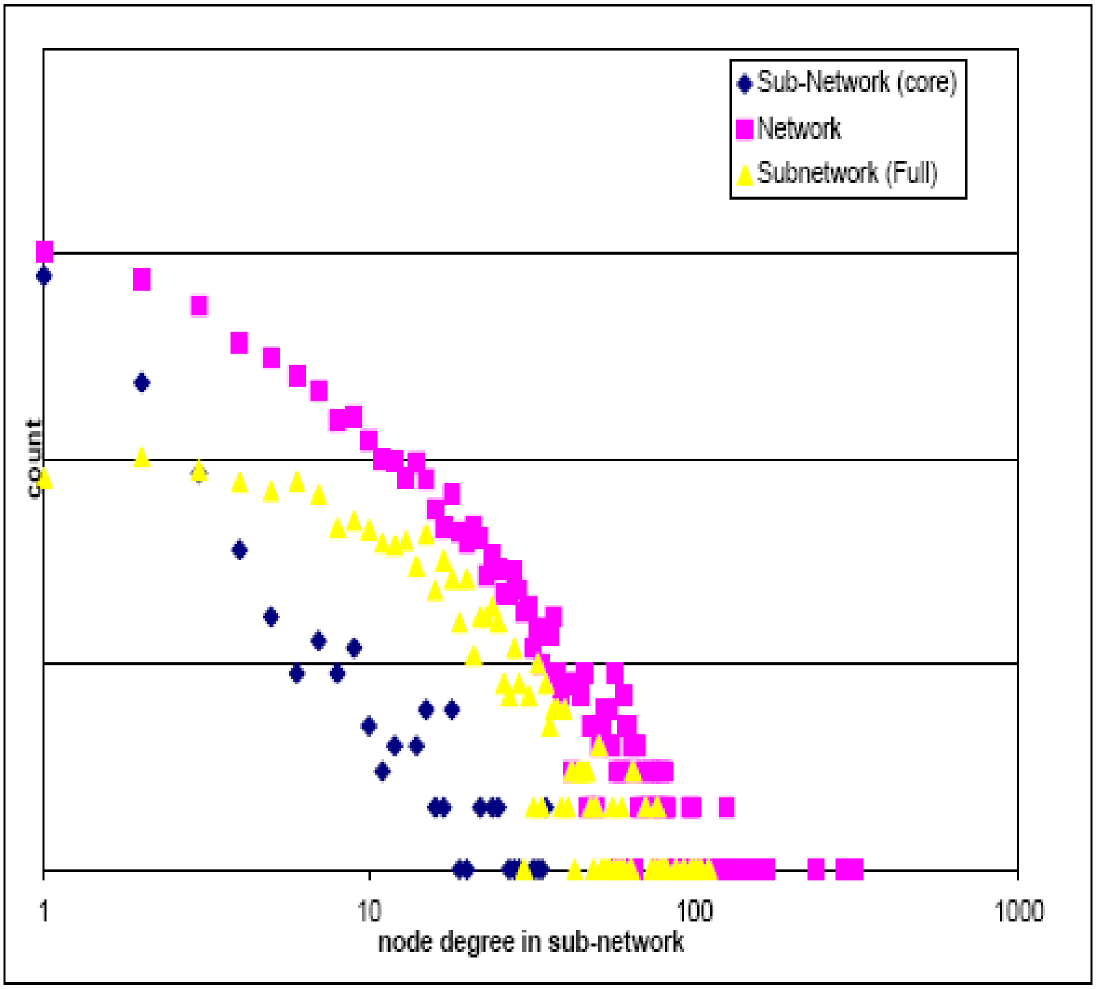


Figure 4.4 Node degree distribution of the sub-networks (core or full) in comparison with the yeast protein interaction network.

The resulting protein interaction sub-network consists of 1,251 seed and non-seed proteins in 1,698 sub-network interactions (including 54 S-S type interactions and 1,644 S-N type protein interactions). This protein interaction sub-network is called a “core sub-network”. The “full sub-network” includes all N-N type protein interactions in addition to the S-S type and S-N type interactions. We plot their node degree frequency distributions in Figure 4.4, where the whole yeast protein interaction network from SBG (labeled “network”) is also shown. As expected, both the network and the sub-network (full) display good “scale-free” property (some nodes act as "highly connected hubs", although most nodes are of low degree). The core sub-network, while perhaps limited in size, begins to show “scale-free like” distribution, although hubs in the sub-network (core) are more distinctively identifiable than overly abundant peripheral nodes by high node degree counts.

We also examined other network features for the core sub-network. The largest connected component of the sub-network consists of 1163 proteins with 1637 interactions. The index of aggregation is 1163/1251=93.0%. The index of expansion as the percentage of S-S type interactions (54) over the core sub-network interactions (1698) is 54/1698=3.18%. The high network index of aggregation here suggests that the core sub-network has high connectivity.

4.6 Analysis of Activated Protein Functional Category Distributions

We first analyzed significantly enriched GO categories among 184 seed proteins. We limited our analysis to level 3 GO categories as previously described. Our result revealed **11** significantly enriched GO biological process, functional categories, and cellular components in response to Grr1 perturbation. After filtering out the general GO categories that have more than 200 ORFs from the whole yeast PPI have been annotated to, only **3** significantly enriched GO categories are left (see table 4.4). The table column header definition is the same as previously defined in the human case study.

Table 4.4. Over/under – represented GO categories among the seed proteins

GO_TERM	ENRICHMENT	(OVER/UNDER)	CONCLUSION
eukaryotic 43S preinitiation complex (C-0016282)	5 (55)	0.0427/0.988	+
lipid transporter activity (F-0005319)	2 (8)	0.0299/0.9979	+
oxidoreductase activity (F-0016491)	16 (184)	0.0007/0.9998	+

The analysis result from the above apparently provides limited information. Thus, a simple ontology-based annotation for global proteomics data offers no significant further understanding of Grr1 function. This is partially due to the fact that the current proteomics techniques are not sensitive enough to capture the whole proteome, especially those proteins with low-abundance, e.g. Grr1. However, we expect to find many abundant proteins whose expression levels are directly or indirectly regulated by Grr1. These proteins may look disparate or isolated in their GO-annotation, but may interact with other proteins and impact the cellular components or functions.

We expanded our protein list by mapping our differentially expressed proteins onto protein interaction networks and including the immediate partners in our analysis. The expanded sub-network included 1251 proteins (184 seeds and 1067 immediate partners). We then mapped these proteins into GO categories and re-analyzed the enriched GO categories using statistical methods previously described. We discovered **53** enriched GO categories including both over- and under-represented GO categories. We applied the same filtering criteria to the 53 GO categories to remove generalized GO categories that contain 200 or more annotations, and obtained **40** enriched GO categories with 15 terms categorized as component terms, 15 categorized as process terms, and 10 categorized as function terms (see table 10). The table column header definition is the same as previously defined in the human case study. Therefore, the application of the GO analysis to the sub-network leads to the extraction of more GO terms overall and more specific GO terms.

Table 4.5. Over/under – represented GO categories among the subnet proteins

GO_TERM	ENRICHMENT	(OVER/UNDER)	CONCLUSION
transcription export complex (C-0000346)	4 (4)	0.0032/1	+
proteasome complex (sensu Eukaryota) (C-0000502)	25 (37)	0/1	++
transcription factor complex (C-0005667)	43 (106)	0.0001/1	++
mitochondrial inner membrane presequence translocase complex (C-0005744)	3 (4)	0.0446/0.9968	+
proteasome regulatory particle (sensu Eukaryota) (C-0005838)	8 (9)	0.0001/1	++
microtubule associated complex (C-0005875)	14 (29)	0.0035/0.999	+
bud (C-0005933)	48 (107)	0/1	++
eukaryotic 43S preinitiation complex (C-0016282)	19 (55)	0.0479/0.975	+
chromatin remodeling complex (C-0016585)	30 (71)	0.0004/0.9998	++
DNA-directed RNA polymerase II\, holoenzyme (C-0016591)	32 (66)	0/1	++
external encapsulating structure (C-0030312)	14 (102)	0.9964/0.0077	-
site of polarized growth (C-0030427)	50 (109)	0/1	++
replisome (C-0030894)	12 (27)	0.0149/0.995	+
cell projection (C-0042995)	19 (36)	0.0002/1	++
pyruvate dehydrogenase complex (C-0045254)	3 (4)	0.0446/0.9968	+
RNA polymerase II transcription factor activity (F-0003702)	42 (93)	0/1	++
receptor signaling protein activity (F-0005057)	8 (13)	0.0041/0.9993	+
amine transporter activity (F-0005275)	1 (33)	0.9999/0.0014	-
organic acid transporter activity (F-0005342)	2 (39)	0.9997/0.002	-
carrier activity (F-0005386)	24 (158)	0.9976/0.0046	-
enzyme activator activity (F-0008047)	20 (56)	0.0304/0.9848	+
lipid binding (F-0008289)	11 (15)	0.0001/1	++
protein transporter activity (F-0008565)	11 (25)	0.0211/0.9928	+
carbohydrate transporter activity (F-0015144)	3 (31)	0.9882/0.041	-
GTPase regulator activity (F-0030695)	26 (63)	0.0016/0.9994	++
aging (P-0007568)	15 (29)	0.001/0.9997	++

morphogenesis (P-0009653)	46 (103)	0/1	++
response to endogenous stimulus (P-0009719)	64 (172)	0/1	++
cell growth (P-0016049)	3 (4)	0.0446/0.9968	+
death (P-0016265)	15 (39)	0.0295/0.9871	+
sexual reproduction (P-0019953)	41 (93)	0/1	++
asexual reproduction (P-0019954)	39 (75)	0/1	++
cell differentiation (P-0030154)	39 (100)	0.0005/0.9998	++
Filamentous growth (P-0030447)	23 (54)	0.0018/0.9993	++
regulation of growth (P-0040008)	3 (3)	0.0136/1	+
regulation of gene expression, epigenetic (P-0040029)	25 (76)	0.0459/0.9737	+
negative regulation of biological process (P-0048519)	53 (155)	0.0021/0.9988	++
non-developmental growth (P-0048590)	14 (28)	0.0023/0.9994	++
regulation of enzyme activity (P-0050790)	13 (25)	0.0021/0.9995	++
reproductive physiological process (P-0050876)	27 (65)	0.0012/0.9995	++

To assess the validity of our analysis, we first determined whether the expanded GO annotation was supported by known Grr1 functions from previous publications.

Grr1 affects many different cellular processes in *Saccharomyces cerevisiae* through its role as a receptor for the SCF ubiquitin ligase[84-86]. In conjugation with this multimeric protein complex, Grr1 serves to target protein substrates for ubiquitylation, an event that ultimately results in the substrates degradation by the 26S proteasome. Currently, there are ten proteins that are thought to be ubiquitylated by the SCF^{Grr1} ubiquitin ligase, each of these proteins playing distinct roles in multiple cellular processes.[86] The cells lacking Grr1 exhibit multiple abnormalities including cell elongation, slow growth on glucose, increased sensitivity to osmotic stress and nitrogen starvation, decreased divalent cation transport, enhanced filamentous growth, defects in sporulation, and slow growth or invariability when combined with amino acid biosynthesis mutants.[84, 87-90] We expect our ontology-driven network enabled approach would capture some of the GO functions through extracting the enriched GO terms directly associated with Grr1 or with the targets of Grr1.

Intriguingly, among the 40 enriched GO categories, 14 GO categories are directly ascribed to Grr1 or at least one of its targets, 10 categories over-represented and 4 under-represented. In figure 4.5, the 10 over-represented GO categories that are directly related to Grr1 or related to targets of Grr1 are shown. The Grr1 protein is known to participate in regulating bud emergence and growth through its role in targeting the Cdc42 effectors Gic1 and Gic2 as well as the cyclins Cln1 and Cln2 for degradation by the 26S proteasome [85, 90-93]. The GO categories “polarized growth”, “bud”, “morphogenesis”, “asexual reproduction”, and “cell projection” are all involved in the elongated bud morphological phenotype of Grr1 knock-out cells. The elongated bud morphology resembles invasive growth for Grr1 knock-out yeast. Therefore, based on the existing evidence from previous publications, the ontology-driven network-enabled analysis approach proves to be not only valid, but also have the potential to drive the generation of the novel hypothesis for future investigations.

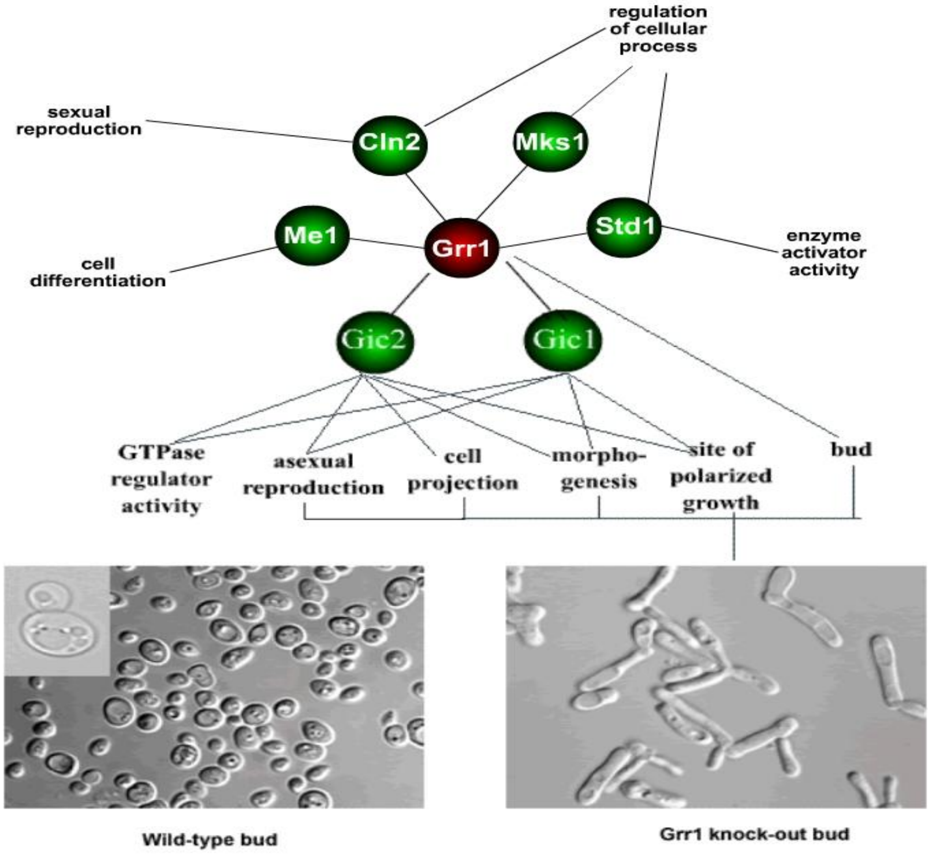


Figure 4.5 Enriched GO categories (partial listing) and yeast bud morphological phenotype. This figure was modified based on Josh's original figure.

4.7 Functional Category Cross-Talks

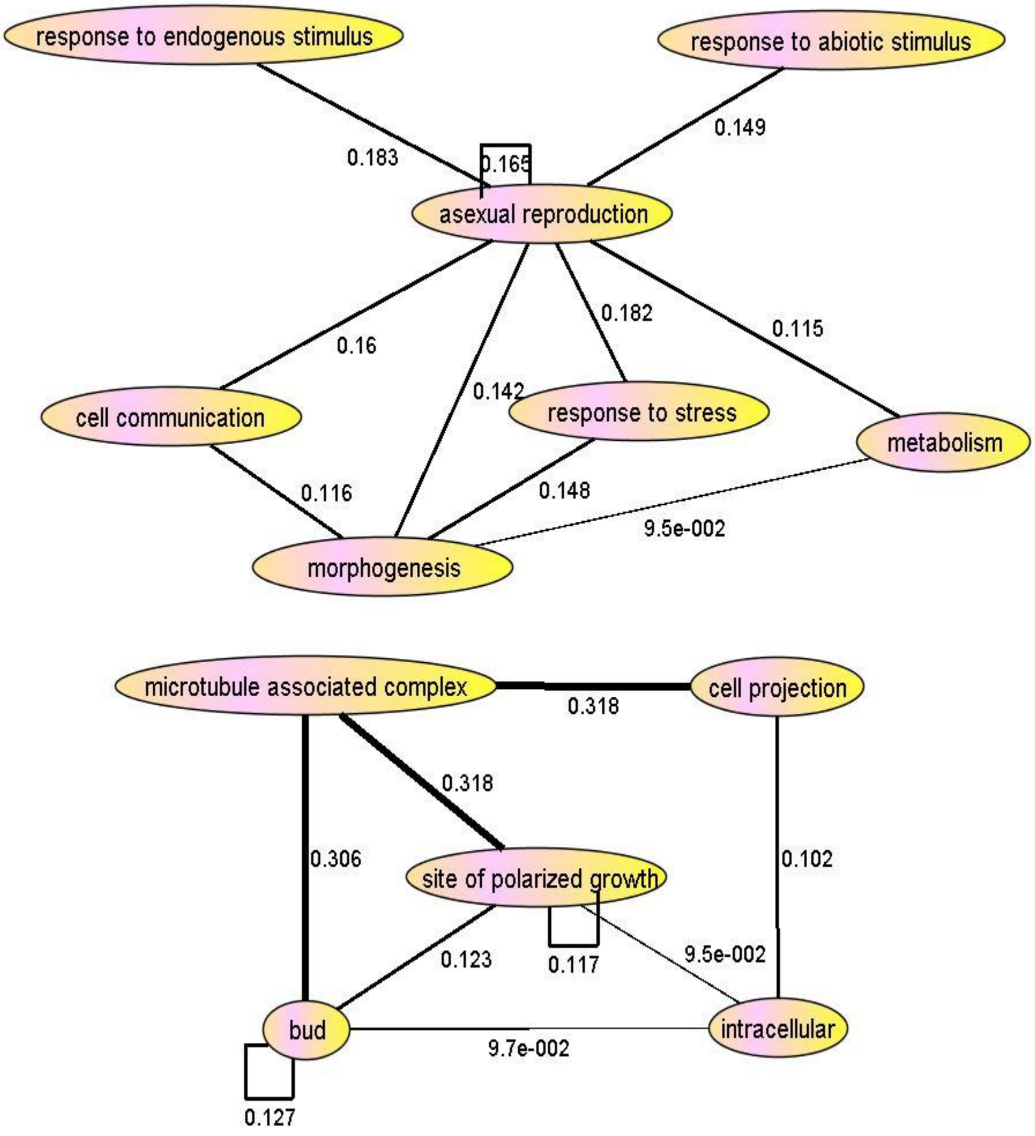


Figure 4.6 Visualization of significantly over-represented GO cross – talk sub-networks related to Grr1 induced morphological change. The partial sub-networks were constructed by seeding the significantly enriched GO cross-talk sub-network by "bud", "cell projection", "site of polarized growth", "asexual reproduction", and "morphogenesis". The top one is for biological process and the bottom one is for

cellular component. The numbers and the thickness both denote the enrichment of the GO cross-talk pairs in the protein-protein interaction sub-network. The larger the number, or the thicker the line, the more enriched the GO pair is.

To investigate how these Grr1-deletion enriched GO categories are functionally associated with each other, we subsequently performed functional category cross-talk analysis (for detailed method, see method section 3.7), and identified 287 significant over/under-represented GO-GO cross-talks (see appendix 4).

In particular, the significantly enriched GO categories discovered previously, i.e. “bud”, “cell projection”, “site of polarized growth”, “asexual reproduction”, and “morphogenesis" are also involved in the significantly enriched GO-GO cross-talk pairs. These cross-talk GO categories are functionally related to the yeast bud morphological phenotype change induced by Grr1 knock-out perturbation. Importantly, some of the GO-GO pairs are highly connected and form the GO-GO interaction subnet for Grr1 (Figure 4.6), implying that Grr1 perturbation may affect some specific biological processes or cellular components through a small core group of proteins. Further more, we also observe that microtube associated complex is highly connected to other GO categories in the GO cross-talk sub-network that are related to Grr1 induced morphological change. This intrigues us since there is no previously known role for Grr1 in microtubule related processes.

Drill-down analysis of the group of proteins involved in the GO cross-talk sub-network might help biologists discover the proteins that significantly contribute to the Grr1 perturbed morphological phenotype change.

4.8 Scoring of Significant Proteins in the Sub-Network

We further performed significant proteins ranking analysis, with the hope of isolating important proteins that contribute to Grr1's possible new function that we discovered earlier by our ontology-driven network-enabled approach.

Based on the connectivity and the confidence of the protein-protein interactions, we ranked significant seed proteins (for detail of the method, see section 3.10). Table 4.6 shows the top-ranked 20 proteins among 184 seed proteins.

Table 4.6 Ranking analysis of the significant proteins. Only 20 top-ranked proteins were listed here due to the space.

Rank	Score	ORF	Gene Symbol	Fold Change (Grr1^{-} vs wt)
1	4.1826	YGL167C	PMR1	0.250
2	3.9453	YPR141C	KAR3	5.970
3	3.8658	YNL298W	CLA4	0.202
4	3.0236	YPL174C	NIP100	0.111
5	2.8646	YOR261C	RPN8	0.33
6	2.8301	YNL233W	BNI4	5.31
7	2.784	YML008C	ERG6	0.129
8	2.7606	YDR155C	CPR1	0.524
9	2.6697	YNL244C	SUI1	4.224
10	2.6495	YKL173W	SNU114	0.409
11	2.6329	YKR054C	DYN1	39.224
12	2.6311	YMR309C	NIP1	13.115
13	2.6219	YJL148W	RPA34	0.129
14	2.5001	YGL055W	OLE1	6.486
15	2.3706	YBL047C	EDE1	0.343
16	2.3508	YBR152W	SPP381	4.658
17	2.328	YDL006W	PTC1	0.229
18	2.3236	YGL112C	TAF6	0.4029
19	2.3079	YLR087C	CSF1	6.4511
20	2.2101	YOR290C	SNF2	0.1812

Later, we isolated the actual proteins from the top ranked protein list that mapped to the GO component term "microtubule associated complex". The analysis revealed that two of the most highly connected proteins, Nip100 and Dyn1. Both proteins also represented most extensively changed proteins in the network: Dyn1 protein levels were observed to increase in the Grr1 mutant ~ 40 fold while Nip100 protein levels were observed to decrease ~10 fold in the analysis. Nip100 is part of the dynactin complex[94] where it is thought to act as a tether for Dynein, encoded by Dyn1. Thus we probed the relationship between Grr1 and Nip100. Figure 4.7 shows protein interaction sub-network seeded by Grr1, Dyn1, and Nip100, where Grr1 connects to

Nip100 through Bzz1 and Tub3. Grr1 connects to Dyn1 through Cdc12 and Pac10. We hypothesized that Grr1's influence on the GO component microtubule associated complex could be possibly through one or more of these bridge proteins such as Bzz1. The biological experiments are being conducted by our biology group to validate this hypothesis.

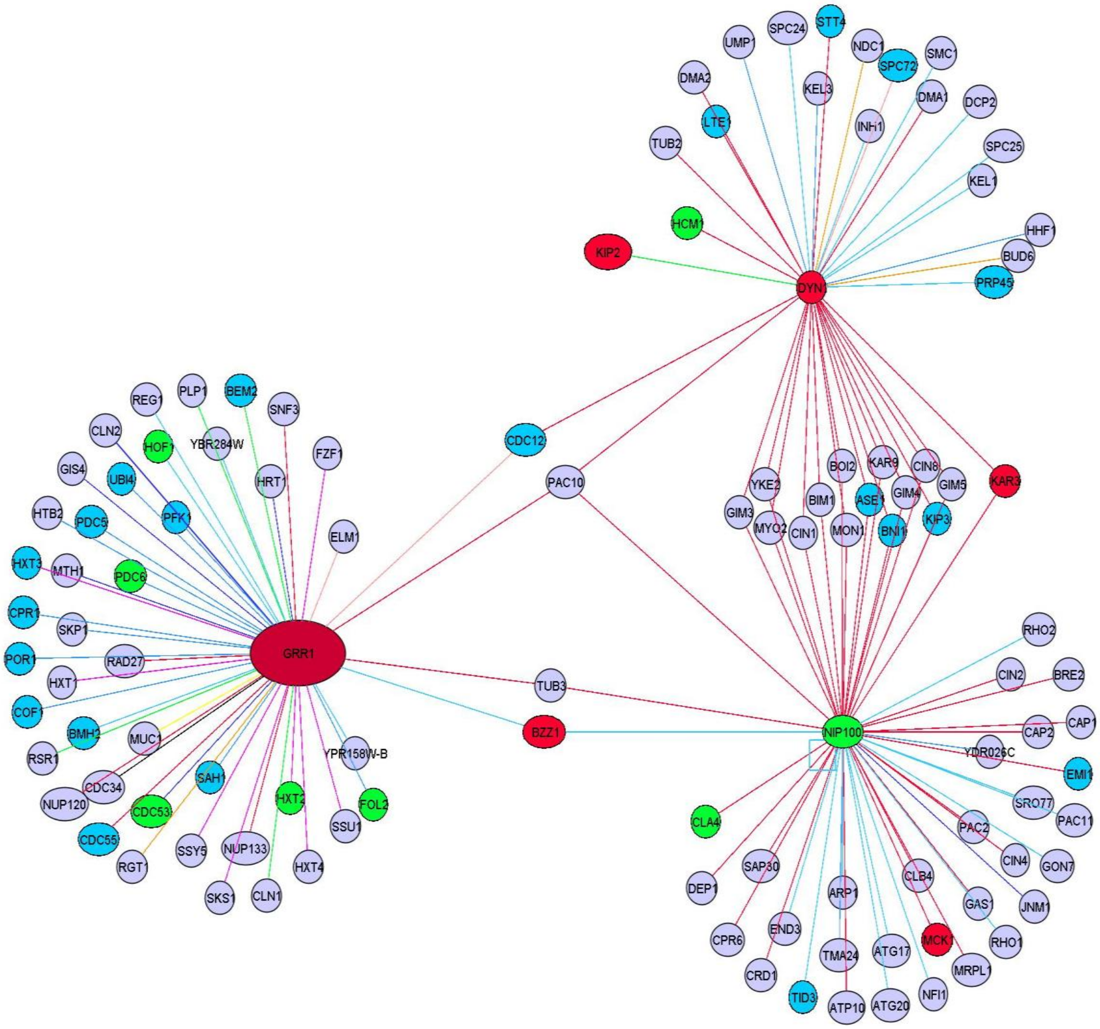


Figure 4.7 Protein interaction sub-network seeded by Grr1, Dyn1, and Nip100. Node colors: Red = protein level > 2 fold, Bright blue = -2>= protein level <=2, Grey = no detection, Green = protein level <-2. Line colors: Red = Synthetic Lethality, Pink = Synthetic Growth Defect, Light Blue = Two Hybrid or Affinity Capture MS, Dark Blue = Reconstituted Complex or Affinity Capture Western, Green = Synthetic Rescue, Purple = Dosage Rescue, Orange = Phenotypic Suppression, Yellow = Phenotypic Enhancement. This figure was provided by Josh Heyen.

Through ranking analysis, we also further validated our ontology-driven network-enabled approach. Kar3, a kinesin-like nuclear fusion protein, is ranked at the second place in the table. It belongs to gene ontology categories “sexual reproduction” (level 3 biological process) and “intracellular” (level 3 cellular component). The third top-ranked protein Cla4, a protein kinase, can be mapped to GO categories “asexual reproduction” at level 4 biological process, and “budding cell apical bud growth”, “cell communication”, “metabolism”, and “morphogenesis” at level 3 biological process. All of the GO categories mapped by these proteins have been shown to be important in Grr1 perturbation induced morphological changes through our ontology-driven and network-enabled approach based analysis.

5. CONCLUSIONS

In the current study, we developed a systems biology approach to analyze the proteomics data. We applied this novel approach to two case studies: human ovarian cancer drug resistance study and yeast Grr1 knock-out study.

The identified differentially expressed proteins formed basic dataset – seed proteins of our case studies. We used the seed proteins to construct our protein-protein sub-network. Then we analyzed the core protein-protein interaction sub-network feature. Both human ovarian cancer drug resistance related and yeast Grr1 knock-out sub-networks showed high connectivity feature. After we mapped the proteins and protein-protein interactions to GO annotations and constructed GO–GO cross-talk sub-networks, we performed statistical testing to find significantly enriched over / under-represented GO categories and GO-GO cross-talk categories. The visualization tools “Spotfire” and “Proteolense” were used to aid in the analysis.

Our approach has been validated in the two case studies by comparing our discoveries with existing findings. Some new insights were obtained.

In the first case study, we observed that cellular physiological process is significantly activated in drug-resistant cell lines, and this response arises from endogenous, abiotic, and stress-related signals. Our studies also showed that cisplatin resistant cell line demonstrated unusually high level of protein-binding activities, and a broad spectrum of cross-the-board drug-binding and nucleotide-binding mechanisms are all activated.

In the second case study, we observed that a subset of significantly over-represented enriched GO categories is highly connected in the GO sub-network, which implies that Grr1 induced morphological phenotype change might be resulted from a small core group of proteins. We hypothesized Grr1's new role in microtubule related processes based on the high connectivity of microtubule associated complex with other GO categories for Grr1's known functions. We further performed ranking analysis of the significant seed proteins based on their connectivities and reliabilities of the interactions in the sub-network. The ranking analysis further validated our findings revealed by the ontology-driven network-enabled approach. These biological discoveries support the significance of developing a common framework of evaluating functional genomics and proteomics data, using networks and systems approaches.

6. DISCUSSIONS

Molecular biology focuses the mechanistic study of biological phenomena. One of its strengths is to concentrate on the actions of a small number of genes without being distracted by the complex biological milieu in which they are found. However, in terms of a series of binary interactions or when pathways become complex and many genes work together, using molecular biology to model function shows its weakness. In this case, more network-level understanding is required. In this study, we showed that the key to interpreting omics data is a systems biology approach, which is both hypothesis-driven and data-driven, with the ultimate goal of integrating multi-dimensional biological signals at molecular signaling network levels. It is important to note that systems biology approach and the traditional molecular biology approach should complement each other in order to achieve a deep understanding of the molecular mechanisms. The systems biology approach is not a replacement of the traditional approach.

In the present study, we described a novel systems biology approach to integrate omics data with both GO annotation and protein interaction networks and its application in two proteomic case studies. The whole proteome of two cellular conditions of yeast or human cells were interrogated using LC-MS/MS. A differentially expressed protein lists were obtained by statistical analyses controlling false discovery rate. We obtained 114 and 184 significantly over- or under-expressed proteins for our two case studies, respectively. The mass spectrometer-based proteomics analysis is one of the major techniques recently developed to examine thousands of proteins simultaneously. Since it directly analyzes the protein level and protein modifications, the mass spectrometer- based proteomics provides more direct explanations for cellular processes involving multiple protein components. However, the current proteomics analysis was unable to detect and quantify an entire proteome and has low sensitivity to the low-abundant proteins such as Grr1, which may play critical roles in many important biological processes.

The large volume of differentially expressed proteins derived from the proteomics and microarray studies provide us the opportunity for investigating the biological function at the systems level. However, the protein lists themselves offers very limited clues to our understanding of the biological processes that underlie cisplatin resistance of human ovarian cancer cells or abnormal phenotype that is associated with Grr1 deletion. Most of the identified significant differentially regulated proteins are not obviously related to the known function of Grr1 or drug resistance. As our first attempt to understand the function of our protein list, we performed ontology-based analysis, which is now widely used in data mining of functional genomic data as well as proteomics data. Gene ontology annotation is an important milestone on possibilities to handle and link biological knowledge with gene profiles identified in functional genomics and proteomics analysis.

Nevertheless, mapping differentially expressed protein list onto ontology provided only limited information to our understanding of the biological processes associated with cellular conditions. For example, our GO annotation analysis of Grr1-deletion affected protein list leads to identification of three enriched GO terms after applying certain filter: eukaryotic 43S preinitiation complex, lipid transporter activity, oxidoreductase activity, none seems to be clearly associated with known function of Grr1. Thus, although GO-based analysis proved to be useful in interpretation of the gene profiling experiments using microarray[95, 96], this technique on its own provide only limited information for our understanding of biological function at the systems level.

Another important approach for interpretation of omics data is network-based analysis. Since most biological characteristics arise from complex interactions between the cellular constitutes such as proteins, mapping changed proteins identified onto protein network will place these proteins in a broader biological context, thereby facilitating the understanding of the structure and function of living cells. In our yeast case study, we mapped our 184 proteins into protein interaction database and ranked the importance of these proteins according to the number of their immediate connectivity and the reliability score calculated using a formula. We hypothesized that the most highly connected proteins in the sub-network may represent proteins that may be mostly directly affected by Grr1 gene deletion. Intriguingly, two of the 20 top-ranked proteins, Nip100 and Dyn1 are genetically and physically connected with each other (Figure 4.7). The physical association of Grr1 with Nip100 is through Bzz1, which appears in our extended protein ranking list. As Nip100 and Dyn1 are a part of the microtubule components, Grr1 may exert its function though its influence on its immediate target Bzz1. This hypothesis warrants a detailed study in the future.

One of the distinct features of our systems biology approach is to bring the gene ontology category concept into the context of protein-protein interaction network and use it for omics data analysis. We hypothesized that the limited number of changed proteins identified through proteomics may actually be connected within Grr1 sub-network. These changed proteins may exert their function by influencing the protein networks that they are involved in. We thus expand our interest of proteins to include those proteins that directly interact with our changed proteins. To understand the biological function of these expanded protein list, we then mapped the protein list onto gene ontology terms and identified significantly enriched GO terms. Through this approach, we found about 40 significantly enriched GO terms by applying a certain filter. Strikingly, 10 of the enriched GO-terms could be ascribed to Grr1 or its target proteins (Figure 4.5) according to previous publications. Thus, our ontology-driven and protein network-enabled approach can not only be used to validate existing knowledge, but also have the potential to generate the hypothesis for future investigation.

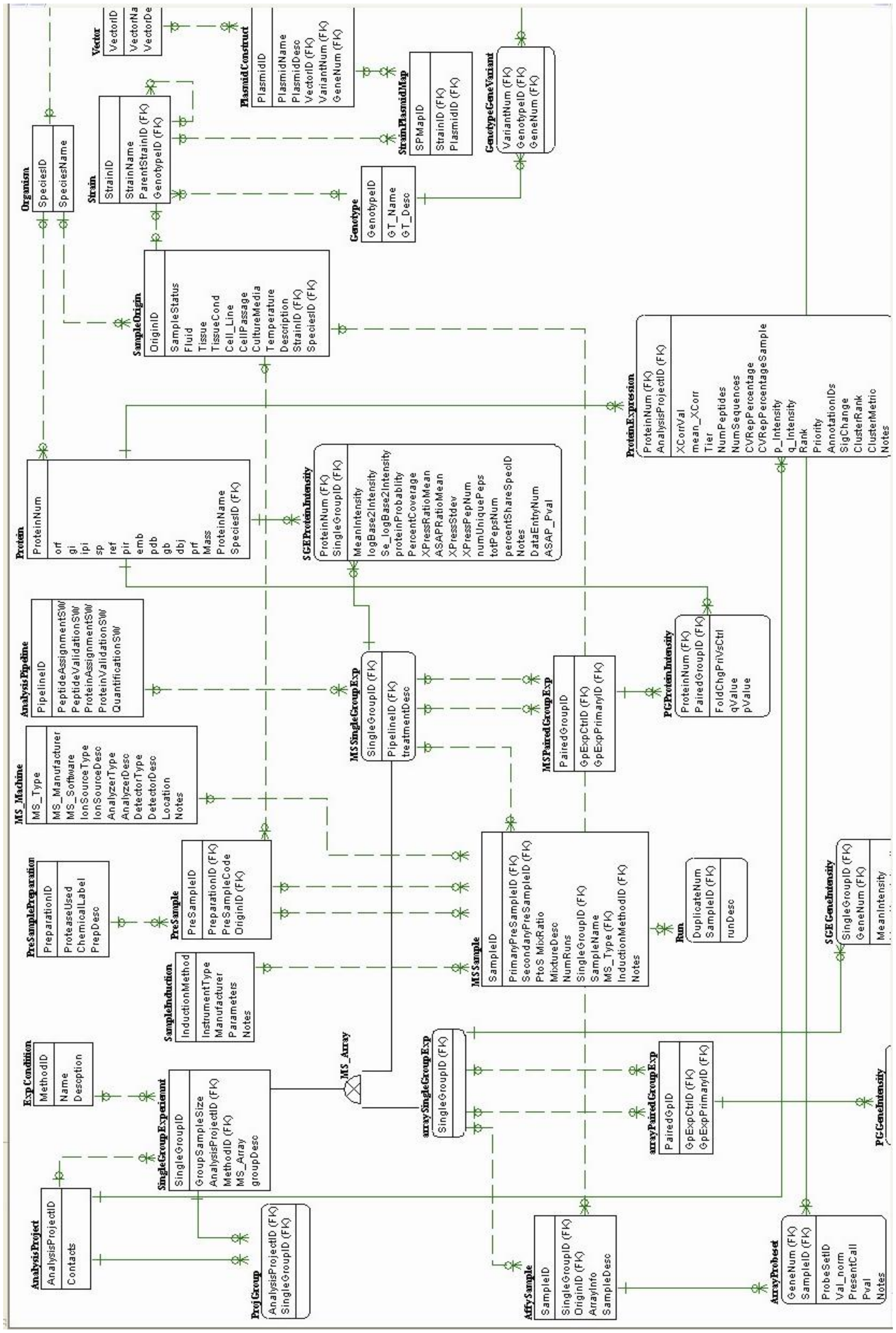
In our approach, we also explored GO-GO cross-talks and identified Grr1-deletion associated GO-GO cross talks. This information further extends our understanding of the connection of multiple processes induced by gene deletion or other stress conditions. We also demonstrated that functional 2-dimensional matrix and protein interaction network visualization tool may significantly facilitate the biologists to form their hypotheses.

Our systems biology approach provides a framework for further improvement in the future. First, our analysis is currently based on proteomics data only. The method described here is readily applicable to microarray data analysis. We expect to gain more in-depth understanding of the Grr1 function by incorporation of published Grr1 microarray data into our analysis. Because the relationship between transcription and translation is likely to vary based on the individual gene/protein, it may not be realistic to expect a high degree of correlation between protein and RNA levels when attempting to correlate dynamic change in RNA with a static picture of proteins. Combination of genomics and proteomics data requires further development of current approach. Second, our network-based analysis focuses on the functional significance of changed proteins through protein-protein interaction analysis. We made no attempt to understand how the changed proteins are regulated by genetic or environmental stress. One of the future directions is to incorporate gene regulatory network analysis in order to identify regulatory relationships among large numbers of genes that form a network representation of the underlying regulatory processes. Finally, our current model needs fine adjustment to provide elegant interpretation of omics data. For example, the validity of ranking model needs further investigation. Drill-down of GO categories analysis may provide further details for interpretation of biological consequences induced by genetic or environmental stresses.

In summary, our ontology-driven network-enabled systems biology approach provides in-depth understanding of cellular functions and creates a robust concept framework for further improvement in the future.

7. Appendices

Appendix 1. ERD diagram for Oracle schema Sysbio. For details, see [21].



Appendix 2. Uniprot ID mappings for 119 differentially expressed seed proteins in ovarian cancer drug resistance study.

UNIPROTID
ANKS1_HUMAN
1433T_HUMAN
1433Z_HUMAN
1433E_HUMAN
1433F_HUMAN
ACTN1_HUMAN
AL3A1_HUMAN
AL3A1_HUMAN
AKAP9_HUMAN
AL1A1_HUMAN
CBX3_HUMAN
DEST_HUMAN
CENPE_HUMAN
CNN2_HUMAN
CRIP2_HUMAN
CRTC_HUMAN
E41L1_HUMAN
FKBP4_HUMAN
6PGD_HUMAN
ABCG1_HUM AN
ACADM_HUMAN
DOCK4_HUMAN
ANXA3_HUMAN
B2MG_HUMAN
DHCA_HUMAN
CAP1_HUMAN
ATPA_HUMAN
ATPB_HUMAN
CU059_HUMAN
CYBP_HUMAN
FA49B_HUMAN
MDHC_HUMAN
KCRU_HUMAN
LPPRC_HUMAN
GALT3_HUMAN
HS70L_HUMAN
HSP76_HUMAN
GANAB_HUMAN
IF4H_HUMAN
HSBP1_HUMAN

HS90B_HUMAN
KAP0_HUMAN
ETFA_HUMAN
PHS3_HUMAN
PP2CG_HUMAN
PPIA_HUMAN
PGK1_HUMAN
PPIB_HUMAN
PDIA1_HUMAN
PDIA6_HUMAN
PARP3_HUMAN
PADI3_HUMAN
RS14_HUMAN
SERA_HUMAN
SODC_HUMAN
SFRS2_HUMAN
SFRS3_HUMAN
INSI1_HUMAN
MYLK_HUMAN
PSB3_HUMAN
PUR6_HUMAN
MYH13_HUMAN
MYL6_HUMAN
MYL6_HUMAN
MYL6_HUMAN
NDK8_HUMAN
PDCD6_HUMAN
O2T35_HUMAN
NDKB_HUMAN
PCNA_HUMAN
PLSI_HUMAN
RL15_HUMAN
TYSY_HUMAN
VINC_HUMAN
UGDH_HUMAN
S10A1_HUMAN
ST1A2_HUMAN
TBA1_HUMAN
TBA3_HUMAN
TCP4_HUMAN
THIL_HUMAN
THIO_HUMAN
SMCA5_HUMAN

TPIS_HUMAN

TBA8_HUMAN

TBAK_HUMAN

STMN1_HUMAN

RPA5_HUMAN

Q12803_HUMAN

O75935_HUMAN

O60486_HUMAN

O14950_HUMAN

Q6IQ55_HUMAN

Q6MZM0_HUMAN

Q6ZSF4_HUMAN

Q5HYM0_HUMAN

Q5VVN3_HUMAN

Q5S007_HUMAN

Q5VU19_HUMAN

Q7Z4V5_HUMAN

Q86XQ2_HUMAN

Q86WH0_HUMAN

Q75MT3_HUMAN

Q9P2M8_HUMAN

Q9NVS0_HUMAN

Q9NZI8_HUMAN

Q9Y2K3_HUMAN

Q9UPT8_HUMAN

Q8TBR1_HUMAN

Q8WU10_HUMAN

ALDOA_HUMAN

CH60_HUMAN

PDIA3_HUMAN

SEC5_HUMAN

PSA6_HUMAN

TBA6_HUMAN

STMN2_HUMAN

RL12_HUMAN

Q96D18_HUMAN

Appendix 3. ORFs for 184 differentially expressed seed proteins in Grr1 knock-out case study

ORF	Fold Change
Q0255	4.15793714718904
YAL038W	0.545852252758714
YAR009C	6.05360443593325
YBL015W	0.328014440120407
YBL016W	8.14232901955471
YBL030C	4.28248520447998
YBL047C	0.34267736598115
YBL088C	2.49526272717271
YBL092W	0.483721610343309
YBR021W	0.157116451158551
YBR078W	0.23105360448864
YBR115C	22.25185704536
YBR136W	4.19593345669184
YBR148W	1.86570263971014
YBR149W	0.25000000018879
YBR152W	4.65804066570279
YBR169C	6.03512014781145
YBR214W	0.203327171879698
YBR218C	8.31792976604813E-02
YBR225W	4.26987061012334
YBR231C	9.54316594964094E-02
YBR233W	12.8927911263819
YBR241C	2.13537469763711
YBR263W	0.147874306878216
YBR272C	2.31265108764462
YBR275C	4.96736103277175
YBR286W	2.96785473982758
YCL011C	2.59830512475747
YCR065W	0.101522842755918
YDL006W	0.228887134956306
YDL019C	9.3956486699021
YDL058W	9.8820593963859
YDL075W	2.26719154500437
YDL127W	5.34195933395087
YDL131W	2.01603150013112
YDL154W	5.66956521767448
YDL176W	0.346494762223967
YDL239C	0.295748613985256
YDR035W	0.419114082837675
YDR058C	4.17638479544648

ORF	Fold Change
YDR081C	0.175600739297833
YDR116C	3.01369863012484
YDR138W	2.44157937145452
YDR155C	0.523771152122715
YDR168W	0.194085027742803
YDR171W	4.26062846561671
YDR177W	0.166358595066621
YDR226W	3.31896375814804
YDR247W	3.68435004836908
YDR351W	21.1090573011355
YDR379W	0.191304347619674
YDR450W	1.97524910005861
YDR464W	20.3099999997752
YDR469W	0.331450044758495
YDR483W	0.240000000159372
YER026C	5.84103511998871
YER042W	6.30314232922456
YER158C	1.91551565055894
YER166W	0.273972603027691
YER176W	0.12628255728794
YER178W	2.10660620275169
YFL003C	0.470655774025158
YFL007W	5.73796369408722
YFR034C	2.19983883943257
YGL003C	0.110667072598724
YGL055W	6.48573742222094
YGL103W	2.07971802652271
YGL112C	0.402900886532899
YGL131C	0.370668815315337
YGL148W	0.277264325355963
YGL151W	0.203327171879698
YGL156W	2.59468170831708
YGL167C	0.249798549363811
YGR004W	12.5138632168835
YGR027C	1.78132111635026
YGR087C	0.228887134956306
YGR132C	0.360000000192903
YGR175C	0.129390018511791
YGR189C	0.29008863801562
YGR203W	9.8383973630905
YGR235C	3.69863013665815
YGR275W	6.88539741255251
YGR284C	0.304990757780443

ORF	Fold Change
YGR288W	14.1988950288631
YHL011C	27.3300000004619
YHL033C	2.45951318287733
YHR104W	9.14445109656317
YHR114W	6.39305445942334
YHR179W	2.63093435531901
YHR198C	6.46253021778323
YIL019W	0.12628255728794
YIL031W	5.01739130432334
YIL041W	2.31952362986739
YIL053W	0.345265042503515
YIL112W	2.50699522170334
YIL143C	8.91870560378892
YIL159W	0.265416928466571
YIR006C	8.86321626617145
YJL005W	3.2126713688849
YJL016W	2.38517324759968
YJL051W	28.3078162776328
YJL148W	0.129390018511791
YJL190C	2.31551734904835
YJL216C	7.52310536012055
YJL218W	0.175600739297833
YJR045C	1.81916371834376
YJR061W	5.96954314750328
YJR066W	23.0050761405552
YKL014C	0.434430958041895
YKL088W	0.306204673567028
YKL173W	0.40890152098861
YKL180W	1.9170591930552
YKL195W	0.394842869081812
YKL209C	8.1319796956819
YKR018C	0.173638516204436
YKR054C	39.2236598928196
YKR057W	0.308407642817507
YKR064W	0.258780037223804
YKR096W	0.177276389876017
YLL007C	3.64222401278672
YLL045C	2.45951318287733
YLL046C	2.20789685726298
YLL057C	4.59352801929727
YLR004C	3.08622078989969
YLR028C	4.13321763210272
YLR080W	0.173638516204436

ORF	Fold Change
YLR087C	6.45107160556459
YLR148W	4.31103948406353
YLR179C	12.5138632168835
YLR191W	0.203045685201405
YLR276C	7.05175600764816
YLR376C	0.286506469458886
YLR422W	0.380221832832661
YLR450W	5.29578856112268
YML008C	0.129390018511791
YML023C	8.44670050774818
YMR038C	0.234289452235187
YMR068W	0.378726832817958
YMR079W	0.339999999966919
YMR096W	0.323475046523754
YMR108W	0.522342689654229
YMR133W	0.249537892567939
YMR145C	3.06832708199654
YMR231W	0.320883101002656
YMR295C	0.31423290183668
YMR309C	13.1146025881953
YMR313C	0.217566478816452
YNL073W	4.1095890411034
YNL079C	0.303703534315121
YNL121C	0.175600739297833
YNL160W	8.4011090582411
YNL218W	0.464296173029536
YNL221C	2.35702717777939
YNL233W	5.31000000022657
YNL241C	0.304990757780443
YNL244C	4.22365988910108
YNL298W	0.201450443110585
YNL313C	14.4269870612683
YNR031C	8.90355330013963
YOL056W	2.57856567271875
YOL059W	2.97340854177039
YOL060C	3.528963333553
YOL081W	0.438552809930963
YOL127W	0.46438538789387
YOR048C	4.43190975024723
YOR129C	3.11039484306185
YOR136W	0.160000000126762
YOR172W	0.145044319229564
YOR187W	0.110905730058634

ORF	Fold Change
YOR191W	10.2233502548427
YOR261C	0.330000000092788
YOR290C	0.181206660197459
YPL007C	27.7043478253533
YPL113C	7.07182320476699
YPL174C	0.110497237621271
YPL231W	0.444474029156303
YPL239W	4.99999999958532
YPL248C	0.149960536676909
YPL255W	12.3475046219541
YPR004C	0.323475046523754
YPR117W	12.2912449348497
YPR134W	0.55600322290172
YPR141C	5.97042513829489
YPR186C	2.90894439963127

Appendix 4 Significantly over/under-represented GO cross-talk pairs for Grr1 knock-out case study

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
F	channel or pore class transporter activity	ion transporter activity	15267	15075
F	ATPase activity\, coupled to movement of substances	ion transporter activity	43492	15075
F	ion binding	ion transporter activity	43167	15075
F	hydrolase activity	ion transporter activity	16787	15075
F	transferase activity	ion transporter activity	16740	15075
F	alcohol transporter activity	ion transporter activity	15665	15075
F	peptide transporter activity	ion transporter activity	15197	15075
F	ion transporter activity	ion transporter activity	15075	15075
C	intracellular organelle	ubiquitin ligase complex	43229	151
C	intracellular	ubiquitin ligase complex	5622	151
C	membrane-bound organelle	ubiquitin ligase complex	43227	151
F	ATPase activity\, coupled to movement of substances	peptide transporter activity	43492	15197
F	ATPase activity\, coupled to movement of substances	channel or pore class transporter activity	43492	15267
F	ATPase activity\, coupled to movement of substances	alcohol transporter activity	43492	15665
F	nucleobase\, nucleoside\, nucleotide and nucleic acid transporter activity	nucleobase\, nucleoside\, nucleotide and nucleic acid transporter activity	15932	15932
F	lyase activity	nucleobase\, nucleoside\, nucleotide and nucleic acid transporter activity	16829	15932
C	intracellular organelle	membrane	43229	16020
C	immature spore	membrane	42763	16020
C	pyruvate dehydrogenase complex	membrane	45254	16020
C	external encapsulating structure	membrane	30312	16020
C	non-membrane-bound organelle	membrane	43228	16020
C	membrane-bound organelle	membrane	43227	16020
P	cellular physiological process	cell growth	50875	16049
C	eukaryotic 48S initiation complex	eukaryotic 43S preinitiation complex	16283	16282
C	eukaryotic 43S preinitiation complex	eukaryotic 43S preinitiation complex	16282	16282
C	non-membrane-bound organelle	eukaryotic 43S preinitiation complex	43228	16282
C	non-membrane-bound organelle	eukaryotic 48S initiation complex	43228	16283
C	intracellular organelle	hydrogen-translocating V-type ATPase complex	43229	16471
F	ion binding	oxidoreductase activity	43167	16491

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
F	transferase activity	oxidoreductase activity	16740	16491
C	RNA polymerase complex	DNA-directed RNA polymerase II\, holoenzyme	30880	16591
F	ion binding	nucleotide binding	43167	166
F	receptor signaling protein activity	nucleotide binding	5057	166
F	carrier activity	nucleotide binding	5386	166
F	electron transporter activity	nucleotide binding	5489	166
F	protein binding	nucleotide binding	5515	166
F	ATPase activity\, coupled to movement of substances	nucleotide binding	43492	166
F	ion transporter activity	nucleotide binding	15075	166
F	transferase activity	nucleotide binding	16740	166
F	cyclase activity	nucleotide binding	9975	166
F	GTPase regulator activity	transferase activity	30695	16740
F	ligase activity	transferase activity	16874	16740
F	ion binding	transferase activity	43167	16740
F	lyase activity	transferase activity	16829	16740
F	hydrolase activity	transferase activity	16787	16740
F	ATPase activity\, coupled to movement of substances	transferase activity	43492	16740
F	ion binding	hydrolase activity	43167	16787
F	ATPase activity\, coupled to movement of substances	hydrolase activity	43492	16787
F	hydrolase activity	hydrolase activity	16787	16787
F	GTPase regulator activity	lyase activity	30695	16829
F	vitamin binding	lyase activity	19842	16829
F	ion binding	ligase activity	43167	16874
C	intracellular organelle	exosome (RNase complex)	43229	178
C	intracellular	exosome (RNase complex)	5622	178
P	regulation of physiological process	sexual reproduction	50791	19953
P	cellular physiological process	sexual reproduction	50875	19953
P	negative regulation of biological process	sexual reproduction	48519	19953
P	regulation of cellular process	sexual reproduction	50794	19953
P	reproductive physiological process	sexual reproduction	50876	19953
P	non-developmental growth	sexual reproduction	48590	19953
P	regulation of cellular process	asexual reproduction	50794	19954
P	localization	asexual reproduction	51179	19954
P	asexual reproduction	asexual reproduction	19954	19954
P	cell differentiation	asexual reproduction	30154	19954
P	reproductive physiological process	asexual reproduction	50876	19954
P	regulation of physiological process	asexual reproduction	50791	19954
P	cellular physiological process	asexual reproduction	50875	19954
P	non-developmental growth	asexual reproduction	48590	19954

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
C	membrane-bound organelle	cell fraction	43227	267
C	intracellular	cell fraction	5622	267
C	intracellular organelle	cell fraction	43229	267
P	reproductive physiological process	cell differentiation	50876	30154
P	cell differentiation	cell differentiation	30154	30154
P	filamentous growth	cell differentiation	30447	30154
P	non-developmental growth	cell differentiation	48590	30154
P	cellular physiological process	cell differentiation	50875	30154
C	intracellular organelle	external encapsulating structure	43229	30312
C	membrane-bound organelle	external encapsulating structure	43227	30312
C	non-membrane-bound organelle	site of polarized growth	43228	30427
C	intracellular organelle	site of polarized growth	43229	30427
C	site of polarized growth	site of polarized growth	30427	30427
P	reproductive physiological process	filamentous growth	50876	30447
P	non-developmental growth	filamentous growth	48590	30447
C	ribonucleoprotein complex	ribonucleoprotein complex	30529	30529
C	intracellular organelle	ribonucleoprotein complex	43229	30529
C	intracellular organelle	Noc complex	43229	30689
F	ion binding	GTPase regulator activity	43167	30695
F	ATPase activity\, coupled to movement of substances	GTPase regulator activity	43492	30695
C	non-membrane-bound organelle	RNA polymerase complex	43228	30880
C	intracellular organelle	transcription export complex	43229	346
C	intracellular	transcription export complex	5622	346
C	non-membrane-bound organelle	transcription export complex	43228	346
C	membrane-bound organelle	transcription export complex	43227	346
F	RNA polymerase II transcription factor activity	nucleic acid binding	3702	3676
F	nucleic acid binding	nucleic acid binding	3676	3676
F	cyclase activity	nucleic acid binding	9975	3676
F	protein binding	nucleic acid binding	5515	3676
F	hydrolase activity	nucleic acid binding	16787	3676
F	translation factor activity\, nucleic acid binding	nucleic acid binding	8135	3676
F	electron transporter activity	nucleic acid binding	5489	3676
P	cellular physiological process	regulation of growth	50875	40008
P	regulation of physiological process	regulation of gene expression\, epigenetic	50791	40029
P	regulation of gene expression\, epigenetic	regulation of gene expression\, epigenetic	40029	40029
P	negative regulation of biological process	regulation of gene expression\, epigenetic	48519	40029
P	cellular physiological process	regulation of gene	50875	40029

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
		expression\, epigenetic		
P	regulation of cellular process	regulation of gene expression\, epigenetic	50794	40029
P	regulation of cellular process	homeostasis	50794	42592
P	cellular physiological process	homeostasis	50875	42592
P	regulation of physiological process	homeostasis	50791	42592
C	intracellular organelle	immature spore	43229	42763
C	membrane-bound organelle	immature spore	43227	42763
C	non-membrane-bound organelle	immature spore	43228	42763
C	membrane-bound organelle	cell projection	43227	42995
C	intracellular organelle	cell projection	43229	42995
F	ion binding	ion binding	43167	43167
F	ATPase activity\, coupled to movement of substances	ion binding	43492	43167
C	pyruvate dehydrogenase complex	membrane-bound organelle	45254	43227
C	membrane-bound organelle	membrane-bound organelle	43227	43227
C	organelle lumen	membrane-bound organelle	43233	43227
C	non-membrane-bound organelle	non-membrane-bound organelle	43228	43228
C	intracellular organelle	non-membrane-bound organelle	43229	43228
C	organelle lumen	intracellular organelle	43233	43229
C	ubiquinol-cytochrome-c reductase complex	intracellular organelle	45285	43229
C	respiratory chain complex III	intracellular organelle	45275	43229
C	pyruvate dehydrogenase complex	intracellular organelle	45254	43229
F	ATPase activity\, coupled to movement of substances	ATPase activity\, coupled to movement of substances	43492	43492
F	cyclase activity	helicase activity	9975	4386
F	ATPase activity\, coupled to movement of substances	helicase activity	43492	4386
F	ion transporter activity	helicase activity	15075	4386
F	transferase activity	helicase activity	16740	4386
P	cellular physiological process	negative regulation of biological process	50875	48519
P	regulation of physiological process	negative regulation of biological process	50791	48519
P	non-developmental growth	negative regulation of biological process	48590	48519
P	regulation of cellular process	negative regulation of biological process	50794	48519
P	regulation of enzyme activity	negative regulation of biological process	50790	48519
F	receptor signaling protein activity	enzyme inhibitor activity	5057	4857
P	localization	non-developmental growth	51179	48590
P	regulation of physiological process	non-developmental growth	50791	48590

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
P	cellular physiological process	non-developmental growth	50875	48590
P	non-developmental growth	non-developmental growth	48590	48590
P	reproductive physiological process	non-developmental growth	50876	48590
P	regulation of cellular process	non-developmental growth	50794	48590
F	ion binding	receptor activity	43167	4872
C	ribonucleoprotein complex	proteasome complex (sensu Eukaryota)	30529	502
C	intracellular organelle	proteasome complex (sensu Eukaryota)	43229	502
C	proteasome regulatory particle (sensu Eukaryota)	proteasome complex (sensu Eukaryota)	5838	502
C	intracellular	proteasome complex (sensu Eukaryota)	5622	502
C	non-membrane-bound organelle	proteasome complex (sensu Eukaryota)	43228	502
F	transferase activity	receptor signaling protein activity	16740	5057
F	ion binding	receptor signaling protein activity	43167	5057
P	cellular physiological process	regulation of enzyme activity	50875	50790
P	regulation of enzyme activity	regulation of enzyme activity	50790	50790
P	regulation of physiological process	regulation of physiological process	50791	50791
P	localization	regulation of physiological process	51179	50791
P	regulation of cellular process	regulation of physiological process	50794	50791
P	regulation of cellular process	regulation of cellular process	50794	50794
P	localization	regulation of cellular process	51179	50794
P	reproductive physiological process	cellular physiological process	50876	50875
P	localization	localization	51179	51179
F	ATPase activity\, coupled to movement of substances	lipid transporter activity	43492	5319
F	hydrolase activity	carrier activity	16787	5386
F	transferase activity	carrier activity	16740	5386
F	ATPase activity\, coupled to movement of substances	carrier activity	43492	5386
F	ion transporter activity	carrier activity	15075	5386
F	alcohol transporter activity	carrier activity	15665	5386
F	channel or pore class transporter activity	carrier activity	15267	5386
F	ion transporter activity	intracellular transporter activity	15075	5478
F	ATPase activity\, coupled to movement of substances	intracellular transporter activity	43492	5478
F	transferase activity	electron transporter activity	16740	5489

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
F	ligase activity	electron transporter activity	16874	5489
F	ion binding	electron transporter activity	43167	5489
F	transferase activity	protein binding	16740	5515
F	cyclase activity	protein binding	9975	5515
C	proteasome regulatory particle (sensu Eukaryota)	intracellular	5838	5622
C	ribonucleoprotein complex	intracellular	30529	5622
C	ubiquinol-cytochrome-c reductase complex	intracellular	45285	5622
C	mRNA cleavage factor complex	intracellular	5849	5622
C	respiratory chain complex III	intracellular	45275	5622
C	histone methyltransferase complex	intracellular	35097	5622
C	mitochondrial inner membrane presequence translocase complex	intracellular	5744	5622
C	immature spore	intracellular	42763	5622
C	transcription factor complex	intracellular	5667	5622
C	pyruvate dehydrogenase complex	intracellular	45254	5622
C	cell projection	intracellular	42995	5622
C	Noc complex	intracellular	30689	5622
C	site of polarized growth	intracellular	30427	5622
C	bud	intracellular	5933	5622
C	eukaryotic 43S preinitiation complex	intracellular	16282	5622
C	external encapsulating structure	intracellular	30312	5622
C	hydrogen-translocating V-type ATPase complex	intracellular	16471	5622
C	microtubule associated complex	intracellular	5875	5622
C	unlocalized protein complex	transcription factor complex	5941	5667
C	intracellular organelle	transcription factor complex	43229	5667
C	immature spore	transcription factor complex	42763	5667
C	membrane-bound organelle	transcription factor complex	43227	5667
C	transcription factor complex	transcription factor complex	5667	5667
C	DNA-directed RNA polymerase II\, holoenzyme	transcription factor complex	16591	5667
C	membrane-bound organelle	mitochondrial inner membrane presequence translocase complex	43227	5744
C	intracellular organelle	mitochondrial inner membrane presequence translocase complex	43229	5744
C	membrane-bound organelle	proteasome regulatory particle (sensu Eukaryota)	43227	5838
C	intracellular organelle	proteasome regulatory particle (sensu Eukaryota)	43229	5838
C	intracellular organelle	mRNA cleavage factor complex	43229	5849

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
C	membrane-bound organelle	mRNA cleavage factor complex	43227	5849
C	intracellular organelle	microtubule associated complex	43229	5875
C	site of polarized growth	microtubule associated complex	30427	5875
C	membrane	microtubule associated complex	16020	5875
C	membrane-bound organelle	microtubule associated complex	43227	5875
C	bud	microtubule associated complex	5933	5875
C	non-membrane-bound organelle	microtubule associated complex	43228	5875
C	cell projection	microtubule associated complex	42995	5875
C	site of polarized growth	bud	30427	5933
C	non-membrane-bound organelle	bud	43228	5933
C	bud	bud	5933	5933
C	intracellular organelle	bud	43229	5933
P	regulation of gene expression\, epigenetic	response to stress	40029	6950
P	asexual reproduction	response to stress	19954	6950
P	negative regulation of biological process	response to stress	48519	6950
P	response to abiotic stimulus	response to stress	9628	6950
P	non-developmental growth	response to stress	48590	6950
P	morphogenesis	response to stress	9653	6950
P	regulation of physiological process	response to stress	50791	6950
P	cell communication	response to stress	7154	6950
P	regulation of enzyme activity	response to stress	50790	6950
P	regulation of cellular process	response to stress	50794	6950
P	cellular physiological process	response to stress	50875	6950
P	response to endogenous stimulus	response to stress	9719	6950
P	negative regulation of biological process	cell communication	48519	7154
P	non-developmental growth	cell communication	48590	7154
P	positive regulation of biological process	cell communication	48518	7154
P	cell differentiation	cell communication	30154	7154
P	regulation of enzyme activity	cell communication	50790	7154
P	response to abiotic stimulus	cell communication	9628	7154
P	cellular physiological process	cell communication	50875	7154
P	regulation of cellular process	cell communication	50794	7154
P	cell communication	cell communication	7154	7154
P	sexual reproduction	cell communication	19953	7154

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
P	metabolism	cell communication	8152	7154
P	morphogenesis	cell communication	9653	7154
P	reproductive physiological process	cell communication	50876	7154
P	regulation of physiological process	cell communication	50791	7154
P	asexual reproduction	cell communication	19954	7154
P	aging	cell communication	7568	7154
P	death	cell communication	16265	7154
P	response to endogenous stimulus	aging	9719	7568
P	cellular physiological process	locomotory behavior	50875	7626
F	cyclase activity	enzyme activator activity	9975	8047
F	ligase activity	translation factor activity\, nucleic acid binding	16874	8135
F	transferase activity	translation factor activity\, nucleic acid binding	16740	8135
P	homeostasis	metabolism	42592	8152
P	asexual reproduction	metabolism	19954	8152
P	regulation of enzyme activity	metabolism	50790	8152
P	regulation of cellular process	metabolism	50794	8152
P	regulation of gene expression\, epigenetic	metabolism	40029	8152
P	regulation of physiological process	metabolism	50791	8152
P	response to abiotic stimulus	metabolism	9628	8152
P	morphogenesis	metabolism	9653	8152
P	negative regulation of biological process	metabolism	48519	8152
P	response to endogenous stimulus	metabolism	9719	8152
P	sexual reproduction	metabolism	19953	8152
P	reproductive physiological process	metabolism	50876	8152
P	non-developmental growth	metabolism	48590	8152
F	ion binding	lipid binding	43167	8289
F	ion transporter activity	protein transporter activity	15075	8565
F	ATPase activity\, coupled to movement of substances	protein transporter activity	43492	8565
F	transferase activity	small protein conjugating enzyme activity	16740	8639
P	regulation of enzyme activity	response to abiotic stimulus	50790	9628
P	cellular physiological process	response to abiotic stimulus	50875	9628
P	sexual reproduction	response to abiotic stimulus	19953	9628
P	asexual reproduction	response to abiotic stimulus	19954	9628
P	response to abiotic stimulus	response to abiotic stimulus	9628	9628
P	reproductive physiological process	response to abiotic stimulus	50876	9628
P	negative regulation of biological process	response to abiotic stimulus	48519	9628
P	non-developmental growth	response to abiotic stimulus	48590	9628
P	cellular physiological process	morphogenesis	50875	9653

TYPE	GO term 1	GO term 2	GO ID1	GO ID2
P	asexual reproduction	morphogenesis	19954	9653
P	non-developmental growth	morphogenesis	48590	9653
P	regulation of cellular process	response to endogenous stimulus	50794	9719
P	regulation of physiological process	response to endogenous stimulus	50791	9719
P	response to endogenous stimulus	response to endogenous stimulus	9719	9719
P	death	response to endogenous stimulus	16265	9719
P	asexual reproduction	response to endogenous stimulus	19954	9719
P	regulation of gene expression\, epigenetic	response to endogenous stimulus	40029	9719
P	negative regulation of biological process	response to endogenous stimulus	48519	9719
P	non-developmental growth	response to endogenous stimulus	48590	9719
P	cellular physiological process	response to endogenous stimulus	50875	9719
F	GTPase regulator activity	cyclase activity	30695	9975
F	transferase activity	cyclase activity	16740	9975

References

1. Smith, J.C. and D. Figeys, *Proteomics technology in systems biology*. Mol Biosyst, 2006. **2**(8): p. 364-70.
2. Chen, J., Yan, Z., Shen, C., Dawn, F., and Wang, M., *A Systems Biology Case Study of Ovarian Cancer Drug Resistance*. JBCB, 2007.
3. Pinto, F.R., et al., *Local correlation of expression profiles with gene annotations--proof of concept for a general conciliatory method.* Bioinformatics, 2005. **21**(7): p. 1037-45.
4. Simpson, R., *Proteins and proteomics: a laboratory manual*. 2003, New York: Cold Spring Harbor Laboratory Press. 926.
5. Lesney, M., *Pathways to the proteome: From 2DE to HPLC.* Modern Drug Discovery, 2001. **4**(10): p. 32-34, 36, 39.
6. Tyers, M. and M. Mann, *From genomics to proteomics.* Nature, 2003. **422**(6928): p. 193-7.
7. Khalsa-Moyers, G. and W.H. McDonald, *Developments in mass spectrometry for the analysis of complex protein mixtures.* Brief Funct Genomic Proteomic, 2006. **5**(2): p. 98-111.
8. Aebersold, R. and M. Mann, *Mass spectrometry-based proteomics.* Nature, 2003. **422**(6928): p. 198-207.
9. Herbert, C.G. and R.A.W. Johnstone, *Mass spectrometry basics*. 2003, Boca Raton: CRC Press. 474 p.
10. Guerrera, I.C. and O. Kleiner, *Application of mass spectrometry in proteomics.* Biosci Rep, 2005. **25**(1-2): p. 71-93.
11. Barker, J., D.J. Ando, and R. Davis, *Mass spectrometry*. 2nd ed. 1999, New York: John Wiley & Sons. xxii, 509.
12. Frohlich, T. and G.J. Arnold, *Proteome research based on modern liquid chromatography - tandem mass spectrometry: separation, identification and quantification.* J Neural Transm, 2006. **113**(8): p. 973-94.
13. Ma, S. and R. Subramanian, *Detecting and characterizing reactive metabolites by liquid chromatography/tandem mass spectrometry.* J Mass Spectrom, 2006. **41**(9): p. 1121-39.
14. Yates, J.R., 3rd, et al., *Method to correlate tandem mass spectra of modified peptides to amino acid sequences in the protein database.* Anal Chem, 1995. **67**(8): p. 1426-36.
15. Old, W.M., et al., *Comparison of Label-free Methods for Quantifying Human Proteins by Shotgun Proteomics.* Mol Cell Proteomics, 2005. **4**(10): p. 1487-502.
16. Keller, A., et al., *Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search.* Anal Chem, 2002. **74**(20): p. 5383-92.
17. Nesvizhskii, A.I., et al., *A statistical model for identifying proteins by tandem mass spectrometry.* Anal Chem, 2003. **75**(17): p. 4646-58.
18. Cottrell, J.S., *Protein identification by peptide mass fingerprinting.* Pept Res, 1994. **7**(3): p. 115-24.

19. Gras, R. and M. Muller, *Computational aspects of protein identification by mass spectrometry.* Curr Opin Mol Ther, 2001. **3**(6): p. 526-32.
20. Yates, J.R., 3rd, *Database searching using mass spectrometry data.* Electrophoresis, 1998. **19**(6): p. 893-900.
21. Yan, Z., et al., *Data Management in Expression-based Proteomics, in Database Modeling in Biology: Theories and Practices. To appear.* 2006.
22. Garwood, K., et al., *PEDRo: a database for storing, searching and disseminating experimental proteomics data.* BMC Genomics, 2004. **5**(1): p. 68.
23. Taylor, C.F., et al., *A systematic approach to modeling, capturing, and disseminating proteomics experimental data.* Nat Biotechnol, 2003. **21**(3): p. 247-54.
24. Garden, P., R. Alm, and J. Hakkinen, *PROTEIOS: an open source proteomics initiative.* Bioinformatics, 2005. **21**(9): p. 2085-7.
25. Ferry-Dumazet, H., et al., *PROTICdb: a web-based application to store, track, query, and compare plant proteome data.* Proteomics, 2005. **5**(8): p. 2069-81.
26. Wilke, A., et al., *Bioinformatics support for high-throughput proteomics.* J Biotechnol, 2003. **106**(2-3): p. 147-56.
27. Cannataro, M., G. Cuda, and P. Veltri, *Modeling and designing a proteomics application on PROTEUS.* Methods Inf Med, 2005. **44**(2): p. 221-6.
28. Yanagisawa, K., et al., *Universal Proteomics tools for Protein Quantification and Data Management -Xome & Mass Navigator.* Genome Informatics, 15th INternational Conference on Genome Informatics, 2004.
29. Weston, A.D. and L. Hood, *Systems Biology, Proteomics, and the Future of Health Care: Toward Predictive, Preventative, and Personalized Medicine.* J. Proteome Res., 2004. **3**(2): p. 179-196.
30. Gene_Ontology, *http://www.geneontology.org/.*
31. Lomax, J., *Get ready to GO! A biologist's guide to the Gene Ontology.* Brief Bioinform, 2005. **6**(3): p. 298-304.
32. Beisvag, V., et al., *GeneTools - application for functional annotation and statistical hypothesis testing.* BMC Bioinformatics, 2006. **7**(1): p. 470.
33. Feng, W., et al., *Development of gene ontology tool for biological interpretation of genomic and proteomic data.* AMIA Annu Symp Proc, 2003: p. 839.
34. Lottaz, C. and R. Spang, *Molecular decomposition of complex clinical phenotypes using biologically structured analysis of microarray data.* Bioinformatics, 2005. **21**(9): p. 1971-8.
35. Khatri, P. and S. Draghici, *Ontological analysis of gene expression data: current tools, limitations, and open problems.* Bioinformatics, 2005. **21**(18): p. 3587-95.
36. Verducci, J.S., et al., *Microarray analysis of gene expression: considerations in data mining and statistical treatment.* Physiol Genomics, 2006. **25**(3): p. 355-63.
37. Draghici, S., *Data Analysis Tools for DNA Microarrays*. Mathematical Biology and Medicine Series. 2003: Chapman & Hall/CRC.

38. Bonferroni, C., *Teoria statistica delle classi e calcolo delle probabilit?* Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze, 1936(8): p. 3-62.
39. Benjamini, Y. and Y. Hochberg, *Controlling the false discovery rate: a practical and powerful approach to multiple testing.* Journal of the Royal Statistical Society. Series B (Methodological) 1995. **57**(1): p. 289-300.
40. Cheverud, J.M., *A simple correction for multiple comparisons in interval mapping genome scans.* Heredity, 2001. **87**(Pt 1): p. 52-8.
41. Zhang, B., S. Kirov, and J. Snoddy, *WebGestalt: an integrated system for exploring gene sets in various biological contexts.* Nucleic Acids Res, 2005. **33**(Web Server issue): p. W741-8.
42. Castillo-Davis, C.I. and D.L. Hartl, *GeneMerge--post-genomic analysis, data mining, and hypothesis testing.* Bioinformatics, 2003. **19**(7): p. 891-2.
43. Shah, N.H. and N.V. Fedoroff, *CLENCH: a program for calculating Cluster ENriCHment using the Gene Ontology.* Bioinformatics, 2004. **20**(7): p. 1196-7.
44. Zhong, S., et al., *GoSurfer: a graphical interactive tool for comparative analysis of large gene sets in Gene Ontology space.* Appl Bioinformatics, 2004. **3**(4): p. 261-4.
45. Khatri, P., et al., *Onto-Tools: an ensemble of web-accessible, ontology-based tools for the functional design and interpretation of high-throughput gene expression experiments.* Nucleic Acids Res, 2004. **32**(Web Server issue): p. W449-56.
46. Martin, D., et al., *GOToolBox: functional analysis of gene datasets based on Gene Ontology.* Genome Biol, 2004. **5**(12): p. R101.
47. Draghici, S., et al., *Onto-Tools, the toolkit of the modern biologist: Onto-Express, Onto-Compare, Onto-Design and Onto-Translate.* Nucleic Acids Res, 2003. **31**(13): p. 3775-81.
48. Ball, C.A., et al., *Saccharomyces Genome Database provides tools to survey gene expression and functional analysis data.* Nucleic Acids Res, 2001. **29**(1): p. 80-1.
49. Barabasi, A.L. and Z.N. Oltvai, *Network biology: understanding the cell's functional organization.* Nat Rev Genet, 2004. **5**(2): p. 101-13.
50. Gavin, A.C., et al., *Functional organization of the yeast proteome by systematic analysis of protein complexes.* Nature, 2002. **415**(6868): p. 141-7.
51. Ho, Y., et al., *Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry.* Nature, 2002. **415**(6868): p. 180-3.
52. Ito, T., et al., *A comprehensive two-hybrid analysis to explore the yeast protein interactome.* Proc Natl Acad Sci U S A, 2001. **98**(8): p. 4569-74.
53. Uetz, P., et al., *A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae.* Nature, 2000. **403**(6770): p. 623-7.
54. Giot, L., et al., *A protein interaction map of Drosophila melanogaster.* Science, 2003. **302**(5651): p. 1727-36.
55. Li, S., et al., *A map of the interactome network of the metazoan C. elegans.* Science, 2004. **303**(5657): p. 540-3.
56. Stelzl, U., et al., *A human protein-protein interaction network: a resource for annotating the proteome.* Cell, 2005. **122**(6): p. 957-68.

57. Rual, J.F., et al., *Towards a proteome-scale map of the human protein-protein interaction network.* Nature, 2005. **437**(7062): p. 1173-8.

58. Brown, K.R. and I. Jurisica, *Online predicted human interaction database.* Bioinformatics, 2005. **21**(9): p. 2076-82.

59. Benson, M. and R. Breitling, *Network theory to understand microarray studies of complex diseases.* Curr Mol Med, 2006. **6**(6): p. 695-701.

60. Wachi, S., K. Yoneda, and R. Wu, *Interactome-transcriptome analysis reveals the high centrality of genes differentially expressed in lung cancer tissues.* Bioinformatics, 2005. **21**(23): p. 4205-8.

61. Sharan, R., et al., *Conserved patterns of protein interaction in multiple species.* Proc Natl Acad Sci U S A, 2005. **102**(6): p. 1974-9.

62. Christie, K.R., et al., *Saccharomyces Genome Database (SGD) provides tools to identify and analyze sequences from Saccharomyces cerevisiae and related sequences from other organisms.* Nucleic Acids Res, 2004. **32**(Database issue): p. D311-4.

63. Issel-Tarver, L., et al., *Saccharomyces Genome Database.* Methods Enzymol, 2002. **350**: p. 329-46.

64. Weng, S., et al., *Saccharomyces Genome Database (SGD) provides biochemical and structural information for budding yeast proteins.* Nucleic Acids Res, 2003. **31**(1): p. 216-8.

65. Alfarano, C., et al., *The Biomolecular Interaction Network Database and related tools 2005 update.* Nucleic Acids Res, 2005. **33**(Database issue): p. D418-24.

66. Bader, G.D., D. Betel, and C.W. Hogue, *BIND: the Biomolecular Interaction Network Database.* Nucleic Acids Res, 2003. **31**(1): p. 248-50.

67. Bader, G.D., et al., *BIND--The Biomolecular Interaction Network Database.* Nucleic Acids Res, 2001. **29**(1): p. 242-5.

68. Calvano, S.E., et al., *A network-based analysis of systemic inflammation in humans.* Nature, 2005. **437**(7061): p. 1032-7.

69. Said, M.R., et al., *Global network analysis of phenotypic effects: protein networks and toxicity modulation in Saccharomyces cerevisiae.* Proc Natl Acad Sci U S A, 2004. **101**(52): p. 18006-11.

70. Seiden-Long, I.M., et al., *Transcriptional targets of hepatocyte growth factor signaling and Ki-ras oncogene activation in colorectal cancer.* Oncogene, 2006. **25**(1): p. 91-102.

71. Motamed-Khorasani, A., et al., *Differentially androgen-modulated genes in ovarian epithelial cells from BRCA mutation carriers and control patients predict ovarian cancer survival and disease progression.* Oncogene, 2006.

72. Higgs, R.E., et al., *Comprehensive label-free method for the relative quantification of proteins from biological samples.* J Proteome Res, 2005. **4**(4): p. 1442-50.

73. Bolstad, B.M., et al., *A comparison of normalization methods for high density oligonucleotide array data based on variance and bias.* Bioinformatics, 2003. **19**(2): p. 185-93.

74. Kersey, P.J., et al., *The International Protein Index: an integrated database for proteomics experiments.* Proteomics, 2004. **4**(7): p. 1985-8.

75. Apweiler, R., A. Bairoch, and C.H. Wu, *Protein sequence databases.* Curr Opin Chem Biol, 2004. **8**(1): p. 76-80.

76. Efron, B., *Large-scale simultaneous hypothesis testing: the choice of a null hypothesis.* JASA, 2004. **99**: p. 96-104.

77. Chen, J., M. Wang, and C. Shen, *An integrated computational proteomics method to extract protein targets for Fanconi Anemia studies.* Proceedings of the 21st Annual ACM Symposium on Applied Computing, Dijion, France. In press, 2006.

78. von Mering, C., et al., *STRING: known and predicted protein-protein associations, integrated and transferred across organisms.* Nucleic Acids Res, 2005. **33**(Database issue): p. D433-7.

79. Sivachenko, A., J. Chen, and C. Martin, *ProteoLens: A Visual Data Mining Platform for Exploring Biological Networks.* BMC Bioinformatics, 2006.

80. Benjamini, Y. and D. Yekutieli, *The control of the false discovery rate in multiple testing under dependency.* Ann. Statist, 2001. **29**(4): p. 1165–1188.

81. Chen, J., C. Shen, and A. Sivachenko, *An Integrated Computational Proteomics Method to Extract Protein Targets for Fanconi Anemia Studies.* Pacific Symposium on Biocomputing, 2006. **11**: p. 367-78.

82. Olivero, O.A., et al., *Preferential binding of cisplatin to mitochondrial DNA of Chinese hamster ovary cells.* Mutat Res, 1995. **346**(4): p. 221-30.

83. Chen, J. and J. Carlis, *Genomic data modeling.* Information Systems, 2003. **28**(4): p. 287-310.

84. Kishi, T., T. Seno, and F. Yamao, *Grr1 functions in the ubiquitin pathway in Saccharomyces cerevisiae through association with Skp1.* Mol Gen Genet, 1998. **257**(2): p. 143-8.

85. Skowyra, D., et al., *F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex.* Cell, 1997. **91**(2): p. 209-19.

86. Spielewoy, N., et al., *Regulation and recognition of SCFGrr1 targets in the glucose and amino acid signaling pathways.* Mol Cell Biol, 2004. **24**(20): p. 8994-9005.

87. Willems, A.R., et al., *SCF ubiquitin protein ligases and phosphorylation-dependent proteolysis.* Philos Trans R Soc Lond B Biol Sci, 1999. **354**(1389): p. 1533-50.

88. Flick, J.S. and M. Johnston, *GRR1 of Saccharomyces cerevisiae is required for glucose repression and encodes a protein with leucine-rich repeats.* Mol Cell Biol, 1991. **11**(10): p. 5101-12.

89. Blacketer, M.J., P. Madaule, and A.M. Myers, *Mutational analysis of morphologic differentiation in Saccharomyces cerevisiae.* Genetics, 1995. **140**(4): p. 1259-75.

90. Loeb, J.D., et al., *Saccharomyces cerevisiae G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway.* Genetics, 1999. **153**(4): p. 1535-46.

91. Kishi, T. and F. Yamao, *An essential function of Grr1 for the degradation of Cln2 is to act as a binding core that links Cln2 to Skp1.* J Cell Sci, 1998. **111 (Pt 24)**: p. 3655-61.

92. Jaquenoud, M., et al., *The Cdc42p effector Gic2p is targeted for ubiquitin-dependent degradation by the SCFGrr1 complex.* Embo J, 1998. **17**(18): p. 5360-73.
93. Barral, Y., S. Jentsch, and C. Mann, *G1 cyclin turnover and nutrient uptake are controlled by a common pathway in yeast.* Genes Dev, 1995. **9**(4): p. 399-409.
94. Kahana, J.A., et al., *The yeast dynactin complex is involved in partitioning the mitotic spindle between mother and daughter cells during anaphase B.* Mol Biol Cell, 1998. **9**(7): p. 1741-56.
95. Eckert-Boulet, N., B. Regenberg, and J. Nielsen, *Grr1p is required for transcriptional induction of amino acid permease genes and proper transcriptional regulation of genes in carbon metabolism of Saccharomyces cerevisiae.* Curr Genet, 2005. **47**(3): p. 139-49.
96. Westergaard, S.L., et al., *Elucidation of the role of Grr1p in glucose sensing by Saccharomyces cerevisiae through genome-wide transcription analysis.* FEMS Yeast Res, 2004. **5**(3): p. 193-204.

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Education

(1). SAS trainings (Session I. SAS programming essentials, Session II. SAS data manipulation) in SAS School, Indianapolis
(2). M.S. in Bioinformatics, Indiana University School of Informatics (GPA: 3.9/4.0)
(3). National Center for Biotech Information Regional Workshop
(4). Microarray data analysis workshop (in Chicago)
(5). M.S. in Cellular Biology from Academy of Military Medical Sciences, Beijing, China
(6). B.S. in Physiology from Nanjing University, Jiangsu Province, China

Professional Experience

- Apr, 2006 ~ current **Business Information Analyst, HSBC**

(1) Database administrator for Access databases: database management, automation processes
(2) Application developer using VBA and VBScript
(3) Data auditor
(4) Reports generator: using VBA, SAS, HTML, and software tools

- Sep, 2005 ~ April, 2006 **Research assistant, Discovery Informatics and Computing Group at Indiana University School of Informatics**

(1) Oracle databases development
(2) Proteomics data analysis and systems biology analysis in SQL, et al.
(3) Programming in Perl and PHP

- Dec, 2004 ~ Aug, 2005 **Research technician, Pediatrics/ Hematology/ Oncology, Indiana University School of Medicine**

(1) Database designer and administrator for Access database
(2) Clinical data analysis in SQL
(3) Microarray data analysis
(4) Programming in VBA, Java, C++, and Matlab

- March, 2000 ~ June, 2001 **Research technician, Urology Department, Indiana University School of Medicine.** Research area: Adenovirus gene therapy for prostate cancer. Cell culturing, virus amplification and purification, plasmid and cosmid construction, numerous assays

- August, 1994 ~ August, 1999 **Assistant Professor, Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences**, Beijing, China. Supervise two technicians. Research area: Preclinical trial, psychopharmacological research, new drug screening and evaluation. Awarded twice by the Institute for excellent work.

- August, 1988 ~ August, 1991 **Assistant lecturer, Traditional Chinese Medicine College**, Jiangxi Province, China.

Computer Science Skills

- Familiar with object-oriented programming concept and programming languages C, C++, Java, SAS, Perl, PHP, JavaScript, Matlab, HTML, XML, Unix/Linux, Excel programming, JCL
- Familiar with database design, Oracle, Access, MySQL database management systems, SQL language
- Familiar with many tools:

  Database related tools: ErWin, Toad for Oracle, Aqua Data Studio, Crystal Reports, Case Studio, MYSQL-Front,

  Application development tools: Eclipse, XML Spy, Komodo

  Multimedia related tools: Dreamweaver, Flash, Fireworks, Illustrator, Adobe Photoshop, Adobe Primere Pro (video editing tool), Adobe Encore (DVD editing tool), SoundForge (sound editing tool), n-Track studio (sound mixing tool), Logo design studio

  Other tools: Office (Excel, Access, PowerPoint, Publisher), Lotus Notes, EndNotes, SSH
- Microarray data analysis (cDNA array and Affymetrix array) and gene annotations
- Knowledge for major biological databases such as NCBI, OWL, Swiss-Prot, PIR, IPI, SGD, GeneOntology
- Data mining techniques such as classification and clustering analysis, association rule extraction
- Familiar with many bioinformatics tools

Hands-on Experience in Biological Research

Primary neural cell culturing, Cell line culturing (293 cells, LnCap, C4-2 cells, PC3 cells, et al); Cell activity assay by MTT assay and Crystal violet assay, X-Gal staining; Adenovirus amplification and purification; Plaque assay for the virus; Protein concentration measurement;Western blot and Northern blot; Making competent bacteria and transformation; Plasmid and Cosmid construction, transfection, PCR and real-time PCR; Plasmid DNA MiniPrep, MidiPrep, MaxiPrep;DNA extraction from tissue, DNA recovery from gel;Radio-labeled ligand receptor binding assay: saturation, inhibition and kinetic assay; Second messenger measurement; RNA microinjection of Xenopus Oocytes; Animal models of depression (rat or mouse forced swimming test, tail suspension test, olfactory bulbectomy for rats, et al).

Projects

- Lookup database management automation. (In VBScript)

- Reports automation processes (in VBA script and VBScript)
- Pediatric leukemia database design, implementation and management (in Access)
- Customized designing an oracle database to store several high-throughput experimental datasets
- Designing and implementing an oracle database to integrate the data downloaded from several public databases (Oracle)
- Lab Ordering Database Design, implementation and management
- Forest Experiment Project Database Design and implementation (in MS Access)
- Student course registration system (in both C and C++), Grocery Distributor System (in C++), Building an AVL tree to generate a word histogram (in C++), Graph for course schedules and shortest time schedule finding (in C++), Thread and Synchronization in Nachos Operating System (in C++), Scheduling in Nachos Operating System (in C++), Memory management in Nachos Operating System (in C++)
- Decision Tree and Neural Network Classification analysis to distinguish 9 different speakers based on 12 Japanese vowel pronunciations (Matlab neural network toolbox and C4.5)
- Clustering analysis of microarray data (Matlab)
- Game character, Name game, Calculator (Java projects)
- Moving fairy (in JavaScript)
- Fun web site (in Flash)

Publications

1) Jake Chen, **Zhong Yan**, Changyu Shen, et al. A Systems Biology Case Study of Ovarian Cancer Drug Resistance. (Accepted for 2007 publication)

2) **Zhong Yan**, Jake Chen, et al. Data Management in Expression-based Proteomics. In: Database Modeling in Biology: Practices and Challenges. (to be published by Springer in 2006).

3) Susanne Ragg, Marc Rosenman, Eve Doucette, **Zhong Yan**, Julie Haydon, Jada Paine, Nadine Lee, Terry Vik, Ketan Mane, Katy Borner.
Data visualization of Multiparameter Information in Acute Lymphoblastic Leukemia Expands the Ability to Explore Prognostic Factors. (Abstract # 554689) 47th ASH Annual Meeting. Aug, 2005

4)**Zhong Yan**, Xiaozhuang Hong et al. The effect of a new anticholinergic drug ^{3}H]tricyclopinate on human fetal cerebral cortex muscarinic receptors. Bull. Acad. Mil. Med. Sci. 1999, 23(1): 35 ~ 7 (Chinese)

5)**Zhong Yan**, Yuhua Chen et al. Effect of MO on animal models of depression. China journal of Chinese meteria medica. 1999, suppl: 81 ~ 2 (Chinese)

6)**Zhong Yan**, Zhenghua Gong et al. Effects of aqueous extracts of detoxified cotton seeds (AEDCS) on the level of mouse spleen lymphocytes cyclic AMP. Bull. Acad. Mil. Med. Sci. 1999, 23(2):136 (Chinese)

7)**Zhong Yan**, Zhipu Luo et al. The effect of equilinol on rat cerebral cortex $GABA_A$ receptors. Bull. Acad. Mil. Med. Sci. 1998, 22(3): 217 (Chinese)

8)**Zhong Yan**, Xiaozhuang Hong et. al. The effect of a new cholinolytic-[^{3}H]Tricyclopinate on human brain muscarinic receptors. Acta. Pharmaceutica. Sinica. 1997, 32(7): 506~10 (Chinese)

9)**Zhong Yan**, Zhipu Luo. Experimental methods of antidepressants. In: Modern experimental methods in pharmacology (I, II). Chief editor: Juntian Zhang. Beijing Medical University and Peking Union Medical College associated press. 1997, 1061 ~ 71 (Chinese)

10)**Zhong Yan**, Zhenghua Gong et al. Effect of oligosaccharide extracted from Morinda officinalis How on corticosterone endangered primary cultured hippocampal neurons. Bull. Acad. Mil. Med. Sci. (accepted before I came to USA)

11)**Zhong Yan**, Zhenghua Gong et al. A new potential anxiolytic: its effects on mitochondrial DBI receptors and on rat elevated plus maze model. (waiting for patent application before I came to USA))