B.Comp. Dissertation (Final Year Project Report)

Inferring Protein Function Module From Protein Interaction Information

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Abstract

Protein complexes are vital in many organisms because they play a crucial part in biological functions, processes and pathways. Recent growth in interactome datasets has spurred developments in methods to use this information to infer protein complexes. These interaction data are often measured in-vitro and contain “hub” proteins that interact with many other proteins. Some of these interactions do not occur at the same space and time in-vivo. The challenge is to group these interactions into subgroups to infer them as protein complexes. Existing methods for making use of such information have been published. However, these methods have low coverage of real protein complexes. In this study, we examine a method to improve one of the existing algorithms for predicting protein complexes, namely the CMC algorithm. We show that our work, the PCF algorithm, produced an improvement in coverage of true protein complexes.

Subject Descriptor:

G.1.0	General
G.2.2	Graph Theory
J.3	Life and Medical Sciences

Keywords:

Protein-Protein Interaction Networks, Iteractome Networks, Maximal Clique Finding, Biological Data, Graph Databases, Protein Complexes.

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1 Introduction

Recent completion of the Human Genome Project has marked a great leap in computational biology. As we move from pre-genomic era to the post-genomic era, more and more emphasis has been put on proteomics – the large scale study of protein structures and functions (Roded et al, 2007). This is in part due to the fact that most genomic sequences can be made available via cheaper sequencing methods and also in part due to the fact that genomic sequences contain no functional information. Moreover, in complex organisms the mechanism of alterative splicing has resulted in poor correlation of mRNA to expressed proteins (Nie et al, 2006). Experiments conducted by biologists, include studies to determine interaction between two proteins. The interactions between proteins in an organism make up the interactome.

Although there are many web-lab methods to detect protein-protein interactions, only a few methods exist for web-lab determination of protein complexes. Protein complexes are a group of two of more proteins which interact with one another in the same space at the same time. Web-lab determination of protein complexes, for example the Tandem Affinity Purification method suffers from inconsistent results due to low reproducibility (Andrzej et al, 2003).

Recently, interactome databases have seen a growth in information, especially after the availability of high-throughput methods. For example, the BioGRID database has now 529,018 proteins with 229,375 interactions collected over 22 organisms. The availability of this information has motivated a number of computational methods to infer protein function and protein function modules. This report is concerned with making use of such interactome information to elucidate protein functional modules, also known as protein complexes.

1.1 Research Background

Many different methods have been used to determine interactome information. For example, two-hybrid systems, affinity capture-MS and co-localization experiments (not exhaustive) have been used. Information derived from such wet-lab experiments has been used in computational analysis and prediction. However, a potential caveat for interpreting such data is that all such wet-lab methods are known to produce some amount of false positives and false negatives. Methods to assess the reliability of protein interactions have been developed (Chua et al, 2008).

As of today, different computational methods have also been available for inferring protein complexes from interactome information. Most of these methods, for example Markov Clustering (MCL), Molecular Complex Detection (MCODE) and Restricted Neighborhood Search Clustering (RNSC), are based on clustering. A recent publication to access the robustness of these methods showed that MCL is most robust in comparison with other methods (Brohee et al, 2006). In addition, another class of complex prediction algorithms, Clustering based on Maximal Cliques (CMC) (Liu et al, 2008) and Protein Complex Predicton (PCP) (Chua et al, 2006) are based on maximal clique finding in interaction networks and has shown to produce good results on certain datasets. In comparison with MCL, the CMC has been shown to produce better matching results (Liu et al, 2008). The primary limitation of CMC is its recall. Like other methods, recall is low on certain datasets such as the MIPS dataset.

1.2 Research Motivation

The motivation for research arises from the fact that low recall values of CMC and other protein complex prediction methods indicates poor coverage of total real protein complexes. In addition, most of the works discussed so far has been concerned with yeast interactome and complexes. While such studies have its uses in elucidating protein function in yeast, very little information can be inferred on human beings. This is primarily due to the reason that human is a much more complex multi-cellular eukaryote as compared to unicellular yeast. We suggest that determining human complexes and hence the possibility of elucidating human protein function can be a useful in understanding some human diseases. It is thus worthy to find methods to improve protein complex coverage and investigate the application of complex finding in the human interactome.

1.3 Research Contributions

We believe that our paper makes the following research contributions:

1. We investigate the causes of low coverage in existing methods like MCL, PCP and CMC.
2. We investigate the possibility of merging interactome datasets across different organisms.
3. We further explain why and which organisms produced better results by merging.

4. We suggest an improvement to CMC that produces better coverage of protein complexes predicted.
5. We also demonstrate that protein complex finding based on interaction network alone may not be sufficient for accurate results. External information may aid to produce better results.

1.4 Datasets

The interactome datasets used in this study were taken from the BioGRID database and the real complexes where taken from the MIPS database. We partitioned the BioGRID interactome dataset according to organisms. In particular, we only used yeast, mouse and human interactome data. The size distribution of human complexes taken from MIPS is shown in figure 1.

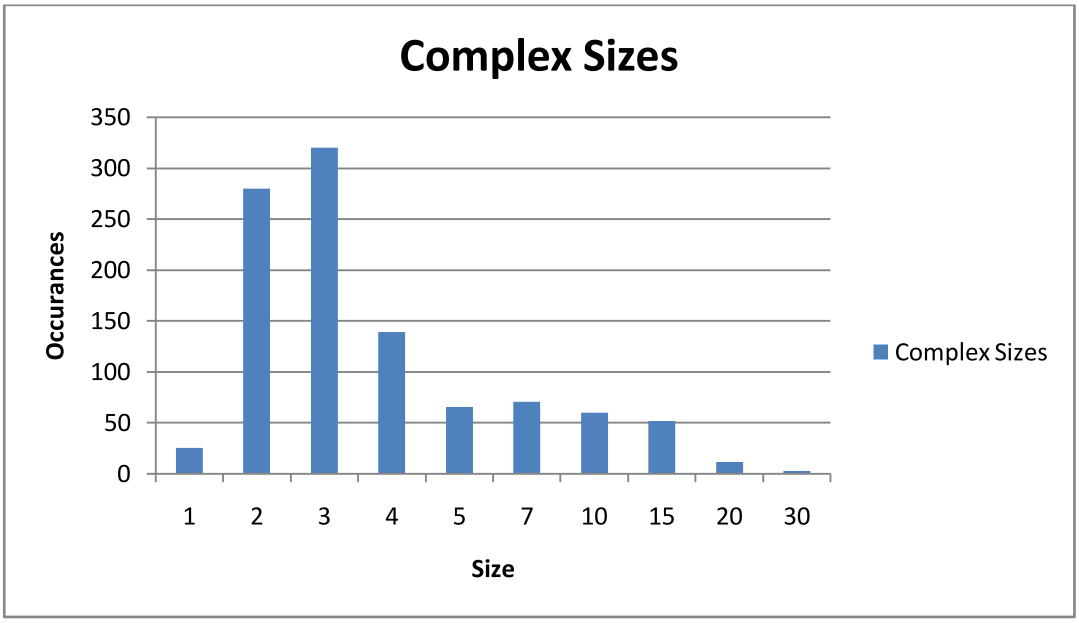

Figure 1. Human complexes size distribution (MIPS database)

1.5 Outline of Report

The report will be organized in two following manner. In section 2, we discuss the MCL, PCP and CMC algorithms together with an analysis of the strengths and limitations of such algorithms. Section 3 discusses other methods that have been considered but were discarded as there were no results. In section 4, we propose methods to improve on current existing approaches. In section 5, we describe the results after validation with complexes obtained from the MIPS database. In section 6, we discuss practical aspects and use of complex prediction. In section 7, we discuss some of the improvements that can be further made.

2 Related Work

Protein complex finding in a given graph, where nodes represent proteins and edges represent interactions between proteins, can be defined as a clustering problem. The aim is to find a cluster of nodes of a graph such that there are many edges within the same cluster but very little edges between two different clusters. In this section, we discuss three of the current available methods - the MCL, PCP and CMC algorithms. In sections 2.1-2.3 we give a brief overview of existing algorithms and in section 2.4, we compare these methods to analyze some of the strengths and limitations inherent to these methods.

2.1 The MCL algorithm

The MCL algorithm has been originally proposed by Van Dongen in a Ph.D thesis (Van Dongen, 2000). The key idea behind the MCL algorithm is that random walks in a graph have a possibility of capturing dense regions in a graph. A random walk is defined as a path on a graph constructed by taking successive random steps. When a number of random k-length paths are chosen, defined at small k (integer), they have a higher probability of beginning and ending in the same dense region. The MCL algorithm consists of two steps, the expansion step and the contraction step. The expansion step allow nodes to visit more neighbors, the contraction step ranks neighbors that are more favorable. The infinite sequence of alternating expansion and contraction make up the Markov Clustering process. The process is stopped when the transition matrix become near idempotent (converges). Van Dongen further explained the methods to obtain clustering information based on the transition matrix when the algorithm halts. The technical details of the algorithm are beyond the scope of this project and will not be discussed. However, we note that the MCL assigns clusters based on the fact that many k-length paths exist within it.

2.2 The PCP algorithm

A novel method for prediction of protein complexes was introduced in 2006. Remarkably, the idea behind the algorithm was simple and mainly based on maximal clique finding and clique merging (Chua et al, 2006). This method was based on the assumption that proteins that form complexes should interact with each other, or in other words, form a complete subgraph. While, this is not always the case, the authors Chua et al used two ideas in an attempt to handle cases where complexes do not form complete subgraphs. In essence, the two interesting ideas are a data preprocessing step and a clique finding and merging step. We describe these two features of the algorithm following:

2.2.1 Weighting of network using FS-weight

FS-Weight can be defined as follows:

$$FS(u,v) = \frac{2|N_u \cap N_v|}{|N_u - N_v| + 2|N_u \cap N_v| + \lambda_{u,v}} \times \frac{2|N_v \cap N_u|}{|N_v - N_u| + 2|N_v \cap N_u| + \lambda_{v,u}}$$

$$where \qquad \lambda_{u,v} = \max(0, n_{avg} - (|N_u - N_v| + |N_u \cap N_v|))$$

N_u represents the direct neighbors of u including u itself. These definitions allow us to see that the FS-weight measures the similarity of two nodes based on how many direct neighbors they share. The impetus of introducing the value $\lambda_{u,v}$ is to penalize protein pairs that have little direct neighbors.

The use of FS-weight to weigh edges of a protein-protein network has been shown to be able to filter unreliable interactions as well as introduce interactions that are predicted to be useful in another independent article (Chua et al, 2006).

2.2.2 Finding and Merging of Cliques

The maximal cliques in a given network can be found via an exhaustive search. Although maximal clique finding in graphs is a known NP-complete problem, the authors have shown that enumerating maximal cliques in sparse graphs like the protein interaction network is not a problem. The author allowed no overlaps between any two maximal cliques by removing the clique that has lower average FS-weight. For any two maximal cliques that are not overlapping, there is a possibility of the cliques being highly interconnected. For this reason, the authors also proposed a method to merge these cliques based on the inter-cluster density score defined as follows:

$$Inter-cluster(C_i, C_j) = \frac{\sum_{u \in Ci-Cj} \sum_{v \in Cj-Ci} w(u,v)}{|C_i - C_j| \cdot |C_j - C_i|}$$

The algorithm halts when no other merging operations can be made for a defined merging threshold

2.3 The CMC algorithm

The CMC algorithm proposed by Liu et al, 2008, extends directly from the PCP algorithm. The similarity with CMC and PCP is in fact the way that both algorithms use clique finding and merging techniques to find protein complexes in a protein-protein network. The differences of the two algorithms lie in different weighting measures as well as different clique merging procedures.

2.3.1 Weighting of network using iterated AdjustCD

$$AdjustCD(u,v) = \frac{2|N_u \cap N_v|}{|N_u| + \lambda_u + |N_v| + \lambda_v}$$

$$where \qquad \lambda_w = \max(0, \frac{\sum_{x \in V} |N_x|}{|V|} - |N_w|)$$

$$w^k(u,v) = \frac{\sum_{x \in |N_u \cap N_v|} (w^{k-1}(x,u) + w^{k-1}(x,v))}{\sum_{x \in N_u} w^{k-1}(x,u) + \lambda_u^k + \sum_{x \in N_v} w^{k-1}(x,v) + \lambda_v^k}$$

$$where \qquad \lambda_y^k = \max\{0, \frac{\sum_{x \in V} \sum_{z \in N_x} w^{k-1}(x,z)}{|V|} - \sum_{x \in N_y} w(x,y)\}\}$$

The iterated AdjustCD can be also similarly defined for FS-weight. In non-iterated scoring, the function on nodes x and y counts the number of shared neighbors between the two nodes. In iterated scoring, the function on two nodes x and y, counts the score of the edges from one node to their shared neighbors which was calculated in the previous iteration. The difference is that instead of using 1 and 0 to represent an edge, the function now uses a score to represent an edge. This method has advantages over non-iterated version because informative scores are used instead of binary numbers. This method has been shown (Liu et al, 2008) to enhance functional homogeneity and localization coherence which are factors strongly correlated to true in-vivo protein interactions and protein complexes.

2.3.2 Merging of Cliques

Clique merging in CMC differs from PCP in a way that overlapping cliques were not discarded if their overlapping score is less than a specified overlapping threshold. The overlapping score is defined as follows:

$$Overlap = \frac{|C_i \cap C_j|}{C_j}$$

If two cliques, C_i and C_j, overlap more than the specified overlapping threshold, it has a possibility of being merged if the inter-cluster score is higher than a specified merging threshold. Otherwise, the lower score cluster is discarded. The inter-cluster score is defined as follows:

$$Inter-cluster(C_i, C_j) = \sqrt{\frac{\sum_{u\in(Ci-Cj)}\sum_{v\in Cj} w(u,v)}{|C_i - C_j|\cdot C_j} \times \frac{\sum_{u\in(Cj-Ci)}\sum_{v\in Ci} w(u,v)}{|C_j - C_i|\cdot C_i}}$$

Where w(u,v) represents a score taken from AdjustCD(u,v) as described above. The meaning behind the inter-cluster score is that cliques that are highly inter-connected have a higher score than cliques that have lower score.

2.4 Analysis of Related Algorithms

At first sight, the maximal clique finding approach of the CMC and PCP looked astounding. This is particularly because there really is no biological evidence of proteins forming the stringent cliquish requirement. One might think that the pre-processing step aided clique detection method by allowing nearly-cliquish clusters to form a complete clique. However, the authors (Liu et al, 2008) discovered that adding new edges do not affect the algorithm so much. Rather, it was due to the clique merging step that allowed the algorithm to capture complexes that were not clique-like.

Although the CMC and PCP algorithms are slightly similar, the tradeoffs between the two algorithms are between precision and recall. The main difference between the two is that PCP disallows overlapping clusters whereas in CMC, overlapping clusters might be retained or merged. In a recent publication, the CMC showed an improvement in recall values on the MIPs dataset as compared to results published by authors of the PCP algorithm (Liu et al, 2008). However, the validation results were not really comparable because the two results were validated using different validation methods. Authors of the CMC algorithm used the Jaccard co-efficient, whereas the authors of the PCP algorithm used the validation criteria defined by Badger et al, 2003.

Jaccard Co-efficient:	Bader's Criteria:
$\frac{\lvert Vs \cap Vc \rvert}{\lvert Vs \cup Vc \rvert}$	$\frac{\lvert Vs \cap Vc \rvert^2}{\lvert Vs \rvert \cdot \lvert Vc \rvert}$

In comparison with MCL, the CMC also performed better as reported by Liu et al, 2008. The reason that MCL did not performed as well was described by Liu et al, due to the fact that MCL also did not allow overlap between clusters. Sources that describe MCL's robustness to noisy data showed that MCL's performance is peak when clusters correspond to regions with many k-length paths within. At this point, there has been no evidence showing a direct correlation between dense k-length paths and protein complexes within interaction network. There has also been very little biological evidence to suggest such a correlation could represent clusters of protein complex.

However, there can be limitations to the CMC algorithm. We list them as follows:

1. There might be a possibility of discarding clusters that are important before merging them with other clusters.
2. Some initial cliques may have already a good representation of a particular protein complex but when merged into a larger cluster, the final result is a false negative.

3 Possible Approaches – A Prelude to PCF

There can be many possible alternatives to infer protein complexes from interaction network. Some of these approaches have been considered and have possibilities of producing results. However, they were discarded either because of complexity issues or because there has been no biological evidence to support these alternatives.

3.1 Frequent Subgraph Mining

Frequent subgraph mining (FSM) has been a hot research topic in data mining. The idea of FSM stems from frequent pattern mining, where a popular apriori algorithm that mines for association rules is derived (Han et al, 2004). Details of the apriori algorithm are beyond the scope of this project and will not be discussed. The aim of FSM is to discover a pattern of subgraph in a database of graphs. In essence, the apriori-based approach consists of only two steps. In the first step, candidate subgraphs are generated. In the second step, the candidate subgraphs are compared to the database of graphs to count the associated statistic. If the subgraph is frequently occurring above a certain threshold, the subgraph is considered a frequent subgraph.

We can make use of FSM to infer protein complexes from interaction networks using the following method:

1. Mine for subgraph patterns in a database of complexes.
2. Filter these mined subgraphs for interesting patterns.
3. Discover these subgraph patterns in an interaction network.
4. Iteratively improve the clusters using some scoring function.

The FSM technique could allow us to see interesting patterns that really occurred in real complexes. However, there are a few complications. Firstly, the process of subgraph isomorphism testing required in step 1 is a known NP-complete problem. Secondly, even if patterns could be mined in an efficient manner, patterns could exist in the network without being a complex itself. Moreover, such a method would be hard to validate since the complex structures were used in the mining process to infer complexes. Lastly, even if validation options could be extended to a different organism, there is no biological evidence that protein complexes are co-related in two different organisms.

3.2 Classification of Cliques in Feature Space

Another possibility could be to improve the CMC algorithm by mapping predicted cliques into a feature space to determine the possibility of being a complex even before the merging process. We propose to stop the merging process if a clique has a high possibility of a protein complex. This might be a solution to the low recall problem mentioned earlier. The main task of such an approach would be to map a set of real complexes as well as randomly generated complexes (negative samples) into a feature space. In order to do that, we need to generate features from a given set of proteins. We selected the following features:

1. Maximum molecular weight protein of proteins in the complex
2. Minimum molecular weight protein of proteins in the complex
3. Average molecular weight of the protein complex
4. Degree of connection of the protein having the maximum molecular weight in the complex
5. Degree of connection of the protein having the minimum molecular weight in the complex
6. Average degree of connection in a protein complex

The features were selected based on our hypothesis that proteins that come together to from complexes obey certain laws of size and connection. We hypothesize that complexes which contain a disproportionate amount of heavy and light weight proteins will cause a change in protein conformation that breaks the complex formation. This means that a randomly generated complex would have very different features as compared to those of a real complex. We created a visualization tool to help us see how protein complexes are locally connected within the complex itself as well as to view these features. A snapshot of the program is provided in appendix A.

In order for classification to work, we require some negative samples to allow us to discriminate a complex from a non-complex. This can be done by generating some random complexes. Generation of random complex is not a trivial task because we had to make sure the random complexes were generated close to the same distribution as that of a real complex. In addition, we also need to provide a random interaction network to account for the negative samples.

We analyzed the set of human complexes provided by the BioGRID database. We found that the number of proteins in a complex followed a gamma distribution and estimated the parameters α=2.5793 and β=1.665 by curve fitting in matlab. By sampling from such a gamma distribution, we created complexes of particular size based on the random number sampled.

We investigated the accuracy of the support vector machine classifier on features extracted from both the positive and negative set. The 10 fold cross validation results were remarkably good, with accuracy reaching 83.97%, and sensitivity of 0.837 on the positive class and 0.842 on the negative class.

These results might mean that our hypothesis were correct. However, a potential caveat arises because the randomly generated complexes might be too far from true negative complexes. A corrected version of the randomization process would require a generation of a random interaction network with the degree of edges that closely resembled a real interaction network. This can be done by mutating edges in a real interaction network such that the newly formed edges are connected to nodes that have similar degree.

Even with a randomly generated interaction network, complications would still arise when one attempts to select random nodes within the network to form a complex. We demonstrate this using an example. Suppose we found that the average connections within a complex follow a particular probability distribution. Ideally, we would like to perform the following steps:

1. Sample from complex size distribution. Let the random number be p.
2. Sample from average connection distribution. Let the random number be q.
3. Proceed to select nodes in a uniformly random manner such that the random complex has size p and have average connection satisfy q.
4. If such a set of nodes are found, add it into collection.
5. Otherwise repeat step 3.

An observant reader will realize that if there are only a few combinations of nodes that satisfy the average connection of q, the algorithm will take a very long time to generate enough negative samples. To make it even more convincing, suppose the network consists of 5,000 proteins. Suppose p=5, then there are $^{5000}C_5$ (2.599×10^{16}) possible combination of complexes. If for a particular q, there are only 5 examples of node sets satisfying this condition, it would not be feasible to expect the random complex generator to halt.

4 Protein Complex Finder – The PCF algorithm

The PCF algorithm was created in an attempt to improve on the works done by the authors of CMC algorithm. In this section, we describe the steps taken to increase the performance of the CMC algorithm.

4.1 Data Pre-processing

4.1.1 Merging Datasets across Organisms

The human interactome is known to be lacking in data. This is because very few web-lab experiments have been conducted to determine human proteins interaction. In contrast, a vast majority of the interaction data belongs to the yeast organism. In our study, we attempted to merge interaction data across two different species as demonstrated in figure 2.

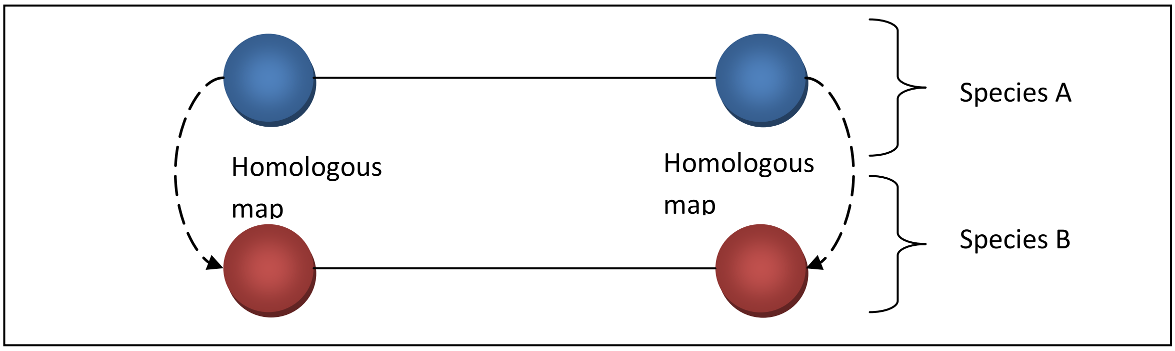

Figure 2. Merging interaction networks by homology

The merging of interaction networks can be done based on homology. There can be two ways to merge interaction networks, resulting in different complexities. We present the naïve method first, as follows:

Given $G_{yeast}<V_{yeast},E_{yeast}>$, $G_{human}<V_{human},E_{human}>$

```
For each $\text{Vertex}_{\text{human}}\ \alpha$
    Find $\text{Vertex}_{\text{human}}\ \beta$ s.t. $\text{Edge}_{\text{human}}(\alpha,\beta)$ does not exist
    For each such $\text{Edge}_{\text{human}}(\alpha,\beta)$
        Let $A = \text{Homolog}(\alpha)$, $B = \text{Homolog}(\beta)$
            If $\text{Edge}_{\text{yeast}}(A,B)$ exist
                Add $\text{Edge}_{\text{human}}(\alpha,\beta)$ to $G_{\text{human}}$
            End if
    End for
End for
```

Algorithm 1. Naïve algorithm for merging interactions between yeast and human

We also define a process of merging with less complexity as follows:

```
Given $G_{\text{yeast}}<V_{\text{yeast}},E_{\text{yeast}}>$, $G_{\text{human}}<V_{\text{human}},E_{\text{human}}>$
For each $\text{Edge}_{\text{yeast}}(A,B)$
    Let $\alpha = \text{Homolog}(A)$, $\beta = \text{Homolog}(B)$
    If $\text{Edge}_{\text{human}}(\alpha,\beta)$ does not exist
        Add $\text{Edge}_{\text{human}}(\alpha,\beta)$ to $G_{\text{human}}$
    End if
End for
```

Algorithm 2. Merging interactions between yeast and human with less complexity

Note that in the above pseudocodes, we did not define the function Homolog. The main problem of this pre-processing step is to define homology.

Homologous proteins can be divided into two subclasses, orthologous and paralogous. Orthologs result from diverging genes between species that derive from a common ancestral organism, maintaining same function. Paralogs result from gene duplication events and have diverged functions. In our study, we are more interested in orthologs, from this point; we use the term homologs and orthologs interchangeably. Tatusov et al, published findings of orthologous proteins called Clusters of Orthologous Groups (COG). Much of their works were based on sequence comparison (Tatusov et al, 2004).

4.1.1.1 The COG database

Using the COG database to infer homologous proteins has its limitations. The challenging problem arises due to the fact that we were using different databases with different unique identifiers. Moreover, the mapping between any two unique identifiers may not be a 1-1 relationship. We demonstrate this complexity using an entity-relationship model as in figure 3.

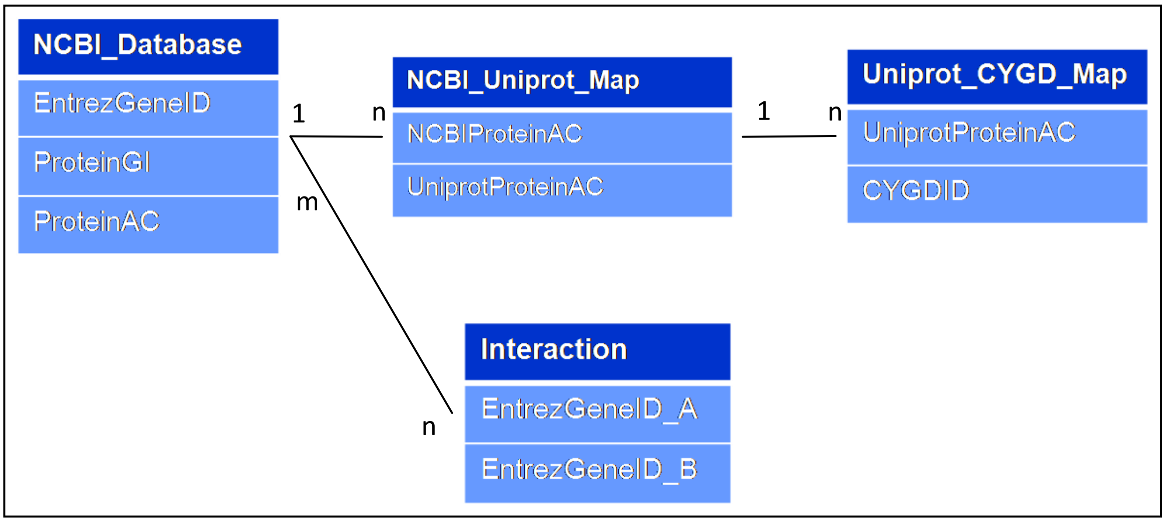

Figure 3. Database entity-relationship model

Essentially, we face two technical problems:

Firstly, the BioGRID interaction data is presented with unique entrezgene_ids obtainsed from the NCBI database. However, COG presents its findings in terms of protein_gi_ids (NCBI) and CYGDids (yeast). The 1-many relationship often results in mapping one edge in the interaction network to many possible edge combinations of a protein_gi_ids.

Secondly, even if we could resolve the ambiguity mentioned earlier, the COG maps one yeast protein to many homologous human protein. Consider $\{\alpha_1...\alpha_n\}$, the set of homologs of A and $\{\beta_1...\beta_n\}$, the set of homologs of B. Another ambiguity arises to decide which of the homologs of A should interact with homologs of B. In practice, we take the Cartesian product to infer that every possible combination is a potential interaction.

Based on the discussion above, it may not be feasible to use the COG database to infer homologous proteins across different species.

4.1.1.2 BLASTp

Due to the limitations described above, alternative methods have been investigated to infer homologous proteins. The problem of finding homologous proteins can be redefined as a database search problem. We formulate the problem as follows. Given a sequence x, we want to find the most similar sequence (allowing some mismatch) in a database. This is a fundamental problem in computational biology and has been explored by many researchers.

In essence, database search methods originated from the smith-waterman algorithm which allows a local alignment of two sequences using dynamic programming in $O(N^2)$ time. Clearly, the use of smith-waterman is not practical in database settings where size of database can be as large as 4 million. This gave rise to approximation methods like FASTA, PatternHunter and BLAST which sacrificed sensitivity for speed. It has been found that BLAST is the de facto standard for sequence comparison by virtue of sensitivity and speed (Anderson et al, 1999). Technical details of BLAST will not be discussed in detail in this paper, however an interested reader would find papers by Altschul et al useful (Altschul et al, 1990). In order to capture homologous information, we used bi-directional BLAST. For example, we BLAST a protein κ from species A against all other proteins in species B. Suppose we found the best match protein η, which is belonging to species B. We then perform BLAST again on protein η to see if protein κ is the best match. If it is, we infer homology, otherwise not.

We now describe the process for inferring homologous proteins using BLASTp

```
For each non-human protein A in non-human network
    Let $\alpha$ be the top hit returned by BLASTp over all human proteins
    Add pair $(A,\alpha)$ to Collection C
End for
For each pair $(A,\alpha)$ in C
    Let B be the top hit of $\alpha$ returned by BLASTp over all non-human proteins
    If B equals to the corresponding A
        Define Homology$(A,\alpha)$
    End If
End for
```

Algorithm 3. Inferring homologous proteins across species

The main advantage of using BLASTp over the COG database is that each human protein is given exactly one yeast homolog. This prevents unnecessary noise introduced by a large number of edge additions. In total, after merging, we have 3 sets of data, listed as follows:

1. Original Human Interaction Network
2. Human & Mouse Merged Interaction Network
3. Human & Yeast Merged Interaction Network

4.1.2 Weighting Interactome Network with Iterated AdjustCD

Merging interactome networks across different species would be helpful if proteins are similar enough to form complexes between two different organisms. The method described in the previous section does not ensure that proteins with that correspond to the first hit in BLASTp will always exist in a complex. This may induce some false positives or false negatives. In order to access to the total reliability of the merged networks, we used the iterated AdjustCD score as described by the authors of the CMC algorithm.

For each set of data after merging, we performed iterated AdjustCD (iterated twice) on the edges and removed edges below a certain threshold as well as add edges above that threshold. We used a threshold of 0.4, as it was reported have best performance in an earlier study (Liu et al, 2008).

4.1.3 Analysis Of Dataset After Pre-processing

In order to ensure that the interactions in the network were coherent before mining for complexes, we used gene ontology (GO) terms to measure function coherence. GO terms are attributes that describe a protein. For example, a protein may belong to particular cellular localization or having a particular function etc. GO terms can be classified as a tree structure where a more specific GO term is a child of a more general GO term. Some of these GO terms may be too general and does not provide useful information with respect to function coherence. Hence, we need to find informative GO terms to describe functional coherence. In essence, there are two methods to do so, which we describe following:

1. Count the number of interactions that share GO terms that are 3 nodes away from the root of the GO term tree structure.
2. Count the number of proteins that belong to a GO term g, such that g has 30 other annotated proteins and each child of g has less than 30 proteins.

We took approach 2 as it was statistically reasonable. Before merging the human interaction network, it originally consisted of 31493 interactions, of which 6487 interactions did have any GO annotation. The remaining 25006 had 14004 interactions with one or more informative GO term, which makes up about 56%. After merging, we obtain 34214 new interactions, of which 8667 have no GO annotation. The remaining 25547 had 16804 interactions with one or more informative GO term, making up about 65%. The percentage increases, when we filtered the network using AdjustCD score, to about 72%. This might suggest that the dataset is good enough to be used for complex prediction.

4.2 Modification of the CMC algorithm

In general, the CMC algorithm suffers in terms of recall in the MIPS complex dataset. The limitations of the CMC have been described earlier (reader might want to review section 2.3.2). In this section, we modify the CMC algorithm to solve one of the limitations. In particular, we want to recover the cliques that might have been discarded and try to merge them if possible.

To recover cliques that were discarded, we first remove interactions that were predicted as protein clusters. Edges can be removed from a network based on the assumption that there will be no overlap in interactions in two different complexes. However, based on observation, this is not true. Instead, certain protein interactions can be observed in more than one complex. If we remove the wrong pair of edges, there is a possibility that we cannot recover cliques representative of protein complexes. We demonstrate this fact using the diagram as in figure 4. (Note that the diagram is only representative of a subgraph of the entire network, which contains many other nodes and edges.)

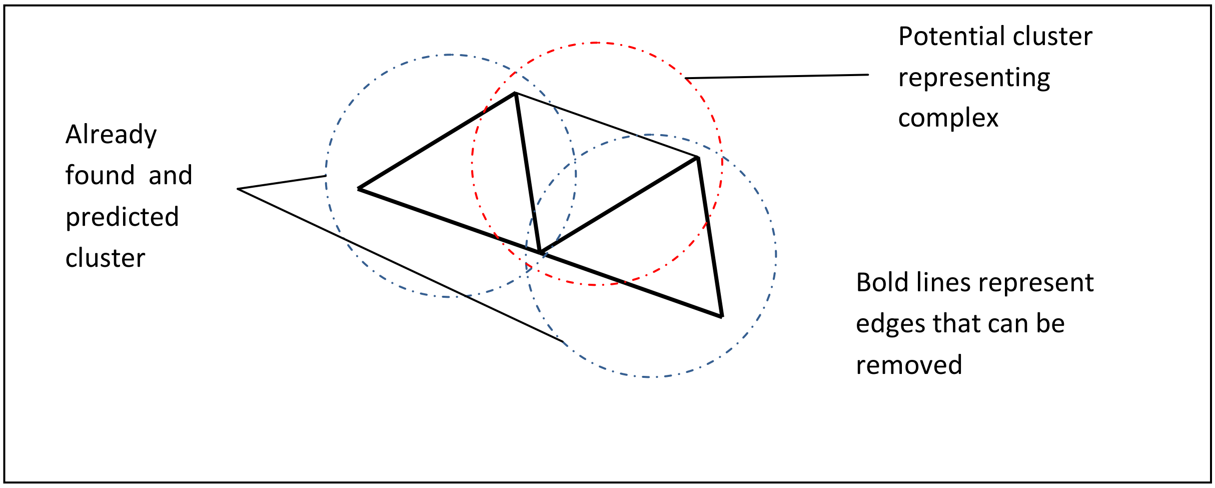
Already found and predicted cluster
Potential cluster representing complex
Bold lines represent edges that can be removed

Figure 4. Pitfalls in removing edges from an interaction network

Hence, we need to find a reasonable method such that important edges are preserved. How can we tell which edges are important? We hypothesize that proteins that belong to many clusters may participate in one or more complexes. Edges connecting to these proteins have a high possibility of becoming part of a clique-like structure, which is critical to the success of our algorithm. Because of this reason, we identify the number of clusters that each protein belongs to. Proteins that belong to clusters that deviate by more than one standard deviation from the mean are considered core proteins. Edges connecting to these core proteins would not be removed.

We modified the CMC algorithm as follows:

```
Given G
Clusters = CMC(G, overlap_thres, merge_thres);
Count, Average, Stdev = 0;
Hashtable core_protein_info;
/* Find how many clusters each protein belongs to */
For each protein p in G
    For each cluster c in Clusters
        If p is in c
            Count++;
        End If
    End For
    Core_protein_info ← (p,count);
End For
<Average, Stdev> = getStatistics(core_protein_info);
/* Remove edges of predicted clusters */
For each cluster c in Clusters
    For each protein p in c
        If (core_protein_info(p) <= Average+1Stdev)
            Remove edges from p to all other p’ in c from G
        End If
    End For
End For
Cluster2 = CMC(G, overlap_thres, merge_thres);
PredictedResults = Cluster2 UNION Cluster;
```

Algorithm 4. Modified CMC algorithm

4.3 Post-processing

In general, we find that the modified CMC algorithm gives much more predicted clusters than actual protein complexes. The predicted set can be reduced by finding the percentage of proteins in a cluster that share informative GO terms (reader might want to review section 4.1.3 for a description of GO terms). For a particular cluster, if the proteins do not share many informative GO terms, we can decide to remove this cluster.

5 Results

In our study, we first compare the CMC algorithm with MCL on human interaction data filtered with iterated AdjustCD as described in section 4.1.2. We also compared the CMC algorithm using a combination of merged interaction data (merging was described in section 4.1.1). We also compared the CMC algorithm with the PCF algorithm (modified CMC described in section 4.2).

We used the same criteria of validation (Jaccard co-efficient described in section 2.4) as proposed by Liu et al, 2008. In addition, we filtered off certain real complexes that were non-existing in the protein interaction network. This is because there is no way to infer complexes if the data does not even exist.

The level of recall allows us to measure the coverage of the algorithms on real complexes. The MCL gave a maximum recall of 12%, whereas CMC gave a much better recall reaching 58% using overlapping and merging thresholds of 0.5. The precision of all the algorithms compared were all lower than 50%. We suggest that the level of precision might not be a good indicator of performance because the list of complexes given in the MIPS database might not be a good representative of total protein complexes in existence. This might be because some have not been found or discovered yet.

When we compare CMC on human and yeast merged network, we found that the maximum recall dropped to 30%. This might be indicative that the merged interactions are representative of noise in the network. When human and mouse merged interaction data were used on CMC, there were no significant change in recall. However, when we use the PCF algorithm, the merged human and mouse merged network gave a 10% higher recall than when run on the original human interaction network. Figure 5 shows the precision vs. recall charts on various settings as described earlier.

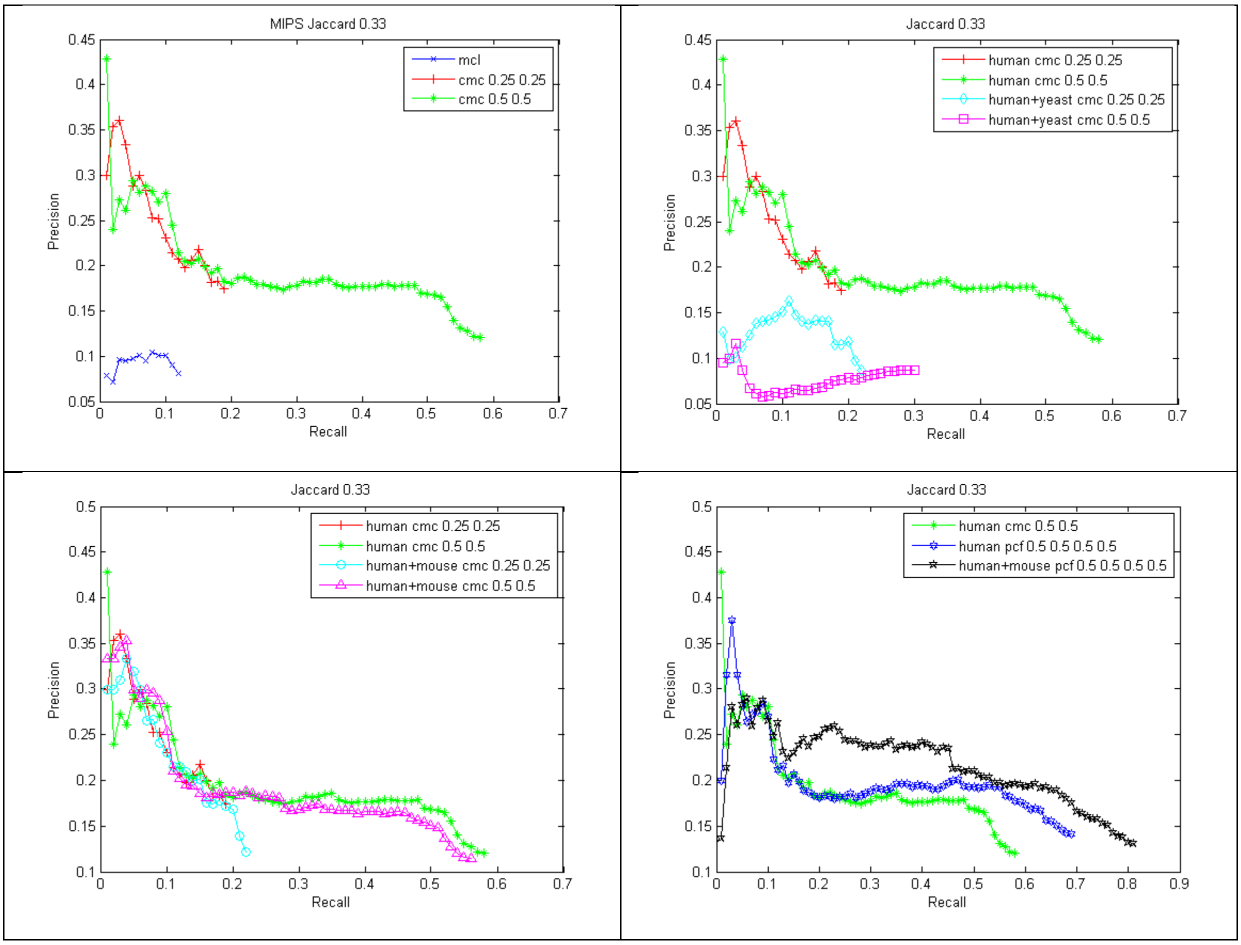

Figure 5. Precision vs Recall Charts. First number represents overlap threshold, second number represents merging threshold. Comparison between MCL and CMC (Top left). Comparison between CMC on human vs. human+yeast (Top right). Comparison between CMC on human vs. human+mouse (Bottom left). Comparison between CMC and PCF (Bottom right).

The human and mouse merged network performed better on PCF probably because human and mouse proteins are highly similar, since they are closer in the evolutionary tree whereas human and yeast are further apart in the evolutionary tree. This might account for the noisy effects of human and yeast merged interaction network on CMC. However, as a side note, we also discover certain areas of our pre-processing that might have resulted in the noisy yeast dataset. In particular, we assumed that when doing bi-directional blast, the top hit always represented something significant. This is not always true. For example, blast results with a low E-value might be representative of occurrence by chance. We did not filter results with low E-values and this might have caused problems. Based on this, we expect mouse dataset to perform better because a blast comparison between mouse and human might contain fewer hits with low E-values than when the comparison was done between yeast and human. We also note that using blast to infer homolog might have its limitations. This is because blast is a local alignment method and a match is found as along as a functional domain is shared. However, there might be other domains which are not common. An ortholog should have all their domains in common. This might present some side-effects which may also affect the human and mouse merged dataset. However, we have not been able to observe this anomaly in our studies.

The precision and recall charts might be hard to interpret because of the fact that some algorithms produced much more predictions than others. Due to this problem, we sorted the predicted clusters according to their average FS-weighted scores and compared the number of matches obtained out of the top nth predictions. We omitted MCL since it was performing much worse than CMC in general. The comparison is shown in table 1. From the table, we find that using overlapping and merging thresholds of 0.25 produced much less predictions than using 0.5 thresholds. This is expected because if the thresholds are small, most of the cliques would be merged, resulting in small number of predictions. When the thresholds are big, the cliques would not be merged so easily, resulting in bigger number of predictions. We also noticed that the PCF performed better consistently.

	Top nth Predictions				
	535	887	1505	1877	4051
Pure Human CMC 0.25 0.25	66/289	-	-	-	-
Human+Yeast CMC 0.25 0.25	61/339	77/339	-	-	-
Human+Mouse CMC 0.25 0.25	64/289	-	-	-	-
Pure Human CMC 0.5 0.5	97/289	149/289	174/289	-	-
Human+Yeast CMC 0.5 0.5	33/339	70/339	132/339	168/339	168/339
Human+Mouse CMC 0.5 0.5	90/289	141/289	168/289	-	-
Human+Mouse PCF 0.5 0.5 0.5 0.5	**126**/289	**174**/289	**227**/289	**242**/289	-

Table 1. Coverage of predicted complexes. Algorithm parameters: first number represents overlapping threshold, second number represents merging threshold. Results: numerator represents number of hits, denominator represents the total real protein complexes

An avid reader might question whether complexes can be discovered by chance since for certain thresholds, CMC predicted up to 4051 predictions when there are only 339 true complexes. We argue that this probability is very small. Suppose there are 5000 proteins, then there are ${}^{5000}C_x$ possible ways to form a complex of size x. The probability to select one true complex by random is given as:

$$\frac{n}{\sum_{i=2}^{m}\binom{5000}{i}}$$

Where n is the number of real complexes, i is the size of a complex and m is the largest possible complex. This number is very small because the denominator values dominate in the equation. For example, suppose the maximum size is 3 and total true complex is 339. Then the probability of 4051 complexes occurring by chance is 6.5x10^{-5}, which is small enough to be considered negligible. The probability becomes even smaller when maximum size increases. Figure 6 shows the probability of choosing 4051 complexes over maximum size.

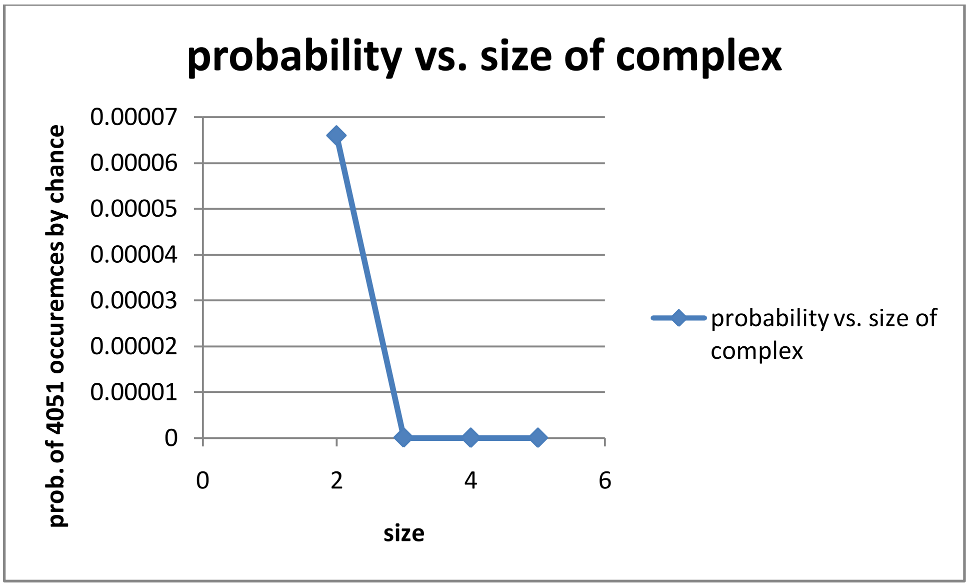

Figure 6. Probability of finding 4051 complexes by random chance

6 Application

The methods presented thus far allow us to infer protein complexes from an interaction network. One may ask, “What is the use of predicting protein complexes?” To show how useful protein complex prediction can be, we discuss possible practical applications of using predicted complexes for biological or clinical analysis. Firstly, some proteins might have no annotations at the moment. Using complex prediction, we can isolate groups of proteins and check if most of them have similar functional annotations. If so, we can guess that the un-annotated protein probably has the same annotation. Secondly, medical researchers who might be interested in looking out for groups of proteins that forms a complex with a particular protein that is inherent to a disease. This can be useful if the researcher has a target protein, he can then proceed to find clusters of proteins containing his target protein. The cluster of proteins found might be useful for drug development and related experiments.

7 Future Work

In this study, we presented a modification to solve one of the limitations of CMC. The modification can be further improved by allowing iterative removal of detected clusters and performing CMC iteratively using different overlapping thresholds and merging thresholds. The other possible improvement could be to use a classifier to stop the algorithm from merging clusters. This method has been discussed under section 3.2, but was difficult to implement due to the fact that negative samples were hard to generate. Instead, negative samples could be obtained by false positives generated by the algorithm which can be mapped to a feature space as previously discussed. However, this method might require robust validation methods because negative samples would be used to train a classifier to which the same negative samples might be subjected to testing. We also propose to re-evaluate our data-preprocessing method, as blast E-values need to be taken into consideration. Finally, we note that validation results were good because we filtered off true complexes that are totally not present in a network. Thus, for such cases, there is no way to infer them using interaction networks. Instead, more information would be required if we want to cover such complexes as well.

In conclusion, we have demonstrated the possibility of merging protein interaction networks based on homology and have shown that it increases the performance of the PCF algorithm. We showed that merged interaction networks of species nearer to each other in the evolutionary tree produced better results whereas merging interaction networks of species further away in the evolutionary tree produced worse results. Results might be further enhanced if errors in our merging operations were corrected. We also showed that the PCF algorithm, which is essentially a modified version of the CMC algorithm, allowed us to obtain higher coverage of complexes. The PCF might be useful for biologists who are interested in studying unknown complexes.

8 References

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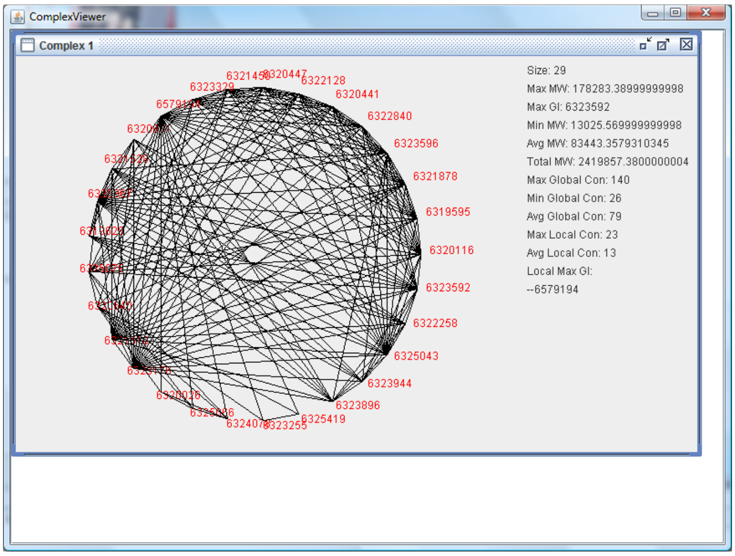
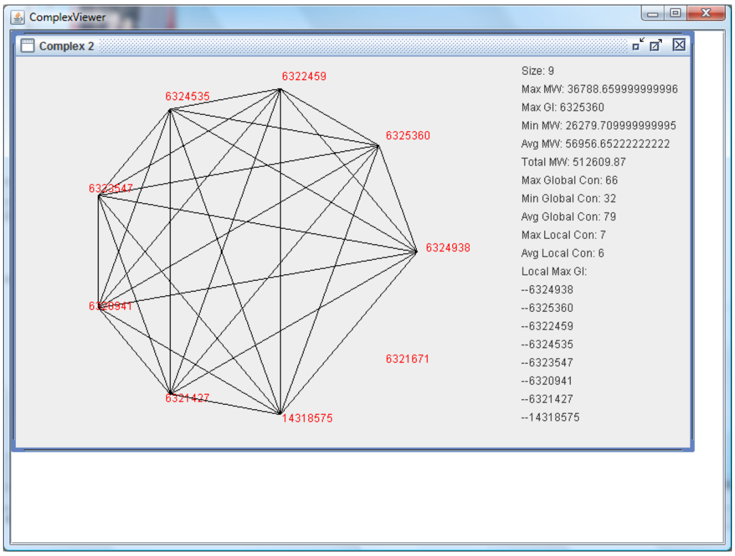
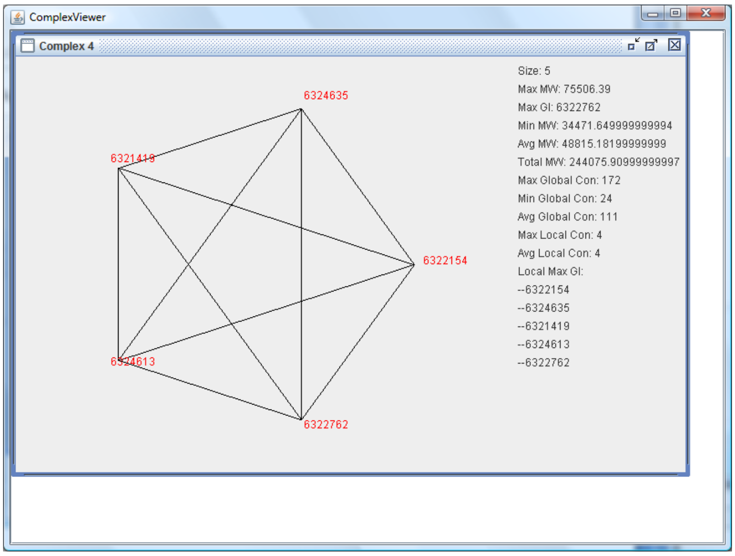
Appendix A - Visualizing complexes and their features



```
Size: 29
Max MW: 178283.38999999998
Max GI: 6323592
Min MW: 13025.569999999998
Avg MW: 83443.3579310345
Total MW: 2419857.3800000004
Max Global Con: 140
Min Global Con: 26
Avg Global Con: 79
Max Local Con: 23
Avg Local Con: 13
Local Max GI:
--6579194
```



```
Size: 9
Max MW: 36788.659999999996
Max GI: 6325360
Min MW: 26279.709999999995
Avg MW: 56956.65222222222
Total MW: 512609.87
Max Global Con: 66
Min Global Con: 32
Avg Global Con: 79
Max Local Con: 7
Avg Local Con: 6
Local Max GI:
--6324938
--6325360
--6322459
--6324535
--6323547
--6320941
--6321427
--14318575
```



```
Size: 5
Max MW: 75506.39
Max GI: 6322762
Min MW: 34471.649999999994
Avg MW: 48815.18199999999
Total MW: 244075.90999999997
Max Global Con: 172
Min Global Con: 24
Avg Global Con: 111
Max Local Con: 4
Avg Local Con: 4
Local Max GI:
--6322154
--6324635
--6321419
--6324613
--6322762
```