

Protein complex prediction via cost-based clustering

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April 5, 2004

Abstract

Motivation: When studying the workings of a biological cell, it is useful to be able to detect known and predict still undiscovered protein complexes within the cell's protein-protein interaction (PPI) network. Such predictions may be used as an inexpensive tool to direct biological experiments. The increasing amount of available PPI data necessitates a fast, accurate approach to biological complex identification.

Results: We have developed the Restricted Neighbourhood Search Clustering Algorithm (RNSC) to efficiently partition networks into clusters using a cost function. We applied this cost-based clustering algorithm to PPI networks of *S. cerevisiae*, *D. melanogaster*, and *C. elegans* to identify and predict protein complexes. We also investigated functional and graph-theoretical properties of known complexes in the MIPS database, and by filtering clusters based on these properties, we attained a high matching rate between filtered clusters and true protein complexes.

Conclusions: Our application of the cost-based clustering algorithm provides a scalable, accurate, and efficient method of detecting and predicting protein complexes within a PPI network.

Availability: The RNSC and data processing code is available upon request from the authors.

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Supplementary Information: Supplementary data is available on the web page:

<http://www.cs.utoronto.ca/~juris/data/ppi04/>

Keywords: protein-protein interaction networks, protein

complexes, graph theory, graph clustering

1 Introduction

Recent developments in the rapidly-expanding fields of network biology and cell biology have resulted in a deluge of protein-protein interaction (PPI) data with accompanying data on protein complexes related to these PPI networks (Uetz *et al.*, 2000; Ito *et al.*, 2000; Ito *et al.*, 2001; Giot *et al.*, 2003; Li *et al.*, 2004; Gavin *et al.*, 2003; Ho *et al.*, 2003). An inevitable consequence of this wealth of data is the need for efficient and accurate automated tools to identify and quantify significant portions of this data. Our method relies on modeling the PPI network with a graph (defined below) and applying principles of both graph theory and gene ontology to identify likely protein complexes with scalable accuracy.

Modeling PPI networks with simple graphs has been used for many applications, one of which is the prediction of protein complexes within the PPI networks (Bader & Hogue, 2003; Pržulj *et al.*, 2004). Protein complexes generally correspond to dense subgraphs in the PPI network, that is, proteins in a given complex are highly interactive with one other (Bader & Hogue, 2003; Pržulj *et al.*, 2004). Previous approaches to graph-theoretic cluster prediction include simple clustering methods such as identification of *k*-cores (Bader & Hogue, 2003) and the highly connected subgraph approach (Hartuv & Shamir, 2000; Pržulj *et al.*, 2004).

We have developed and applied the Restricted Neighbourhood Search Clustering algorithm (RNSC), which partitions the network's node set into clusters based on

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a cost function that is assigned to each partitioning. We then filtered the RNSC output so that only clusters which share characteristics of known protein complexes are considered. This method was applied to the same four *S. cerevisiae* PPI networks discussed in (Pržulj *et al.*, 2004), as well as two *D. melanogaster* PPI networks (Giot *et al.*, 2003) and a *C. elegans* PPI network (Li *et al.*, 2004). Our criteria for filtering the clusters were cluster size, cluster density, and functional homogeneity, all of which are discussed later in this paper. We compared the results of our method with known yeast protein complexes (Mewes *et al.*, 2002) and found that with appropriate choices for filter cutoff values, high matching rates along with large cluster sample sizes can be achieved.

For a deeper look at the application of graph theory to cellular biology, see (Barabási & Oltvai, 2004; Newman, 2003; Albert & Barabási, 2002; Strogatz, 2001; Pržulj, 2004). The last one focuses specifically on PPI networks.

2 System and Methods

Our protein complex prediction method relies on modeling PPI data as *graphs* (or *networks*). A graph $G = (V, E)$ is a set V of *nodes* (or *vertices*), representing proteins, and a set E of *links* (or *edges*), representing interactions between pairs of proteins. Each edge joins two nodes. We also use $G(V)$ to denote the set of nodes V of G (West, 2001).

We used four *S. cerevisiae* PPI networks originating from (von Mering *et al.*, 2002) comprising 2,455, 11,000, 45,000, and 78,390 interactions. We call these networks Y_{2k} , Y_{11k} , Y_{45k} and Y_{78k} respectively, the smallest one containing high confidence interactions only and the larger ones having an increasing number of lower confidence interactions. We used two *D. melanogaster* PPI networks, one derived from the entire fruitfly network of interactions given in (Giot *et al.*, 2003), and one derived from those interactions with confidence greater than 0.5; these have 20,007 and 4,637 interactions respectively, and we call these networks F_{20k} and F_{4k} . We also used a *C. elegans* PPI network, W_{5k} , consisting of 5,222 interactions (Li *et al.*, 2004) (also see (King *et al.*, 2004)). To analyze these networks, we first clustered them using the RNSC algorithm, then we filtered the results based on cluster size, density, and functional homogeneity.

This two step approach preserves only those clusters which have properties more likely present in true biological complexes.

To evaluate how effective our algorithm is for detecting protein complexes, we compared the filtered clusters of the yeast PPI networks with known protein complexes in the MIPS yeast complex database (Mewes *et al.*, 2002). Whether or not a given cluster is deemed to match a given MIPS complex depends on the matching criteria detailed below.

2.1 Clustering

The bulk of the computation time was spent clustering the PPI networks using RNSC algorithm, which is described in Section 2.6. Results included very small clusters and clusters which were either insufficiently dense, or whose component proteins had too weak a concentration in a single functional group. To achieve a high prediction rate, we discarded these clusters. The appearance of these clusters is not a problem with the algorithm, rather it is a product of the fact that the networks are very sparse and that the algorithm partitions the network, and thus each protein *must* be assigned to a cluster (see Section 2.6).

2.2 Cluster Size

The notion that we want to discard small clusters comes from two ideas: first, any overlap proportion between a small predicted complex and a known complex is more likely to be by chance than the same overlap proportion involving a larger predicted complex; second, small known complexes frequently have low density in current PPI networks and are therefore difficult to detect using a clustering algorithm. We experimentally determined a lower bound for a cluster size and discarded all predicted complexes with size below this lower bound. The size bound is dependent on the PPI network in question.

2.3 Cluster Density

Protein complexes are wed to the ideal property that their proteins have high interaction rates with each other. We therefore consider lower-density clusters to be less likely to correspond to known protein complexes. By discarding

clusters whose densities lie below a certain threshold, we can increase the prediction rate of our algorithm.

2.4 Functional Homogeneity

It has been observed that known protein complexes often have high functional homogeneity (Bu *et al.*, 2003; Pržulj *et al.*, 2004). That is, a large proportion of proteins within a known complex likely belongs to a common functional group. This is also true for dense regions of PPI networks (Bu *et al.*, 2003; Pržulj *et al.*, 2004). The functional homogeneity P -value is the probability that a given set of proteins is enriched by a given functional group merely by chance, following the hypergeometric distribution. The P -value for a cluster C and a functional group F is

$$P = 1 - \sum_{i=0}^{k-1} \frac{\binom{|F|}{i} \binom{|V|-|F|}{|C|-i}}{\binom{|V|}{|C|}} \quad (1)$$

where C contains k proteins in F , and the entire PPI network contains $|V|$ proteins (also used in (Bu *et al.*, 2003; Pržulj *et al.*, 2004)). We consider the P -value of a cluster to be its smallest P -value over all functional groups. Functional group data is derived from (von Mering *et al.*, 2002) for the yeast networks.

We discarded all clusters with P -value above a given, experimentally derived threshold (see Section 3.1). Although our model of functional homogeneity is very simple, the fact that known protein complexes have low P -values indicates that taking such an approach to evaluate PPI network clusters as potential protein complexes will be effective. Sensible cutoffs for the cluster P -values range from 10^{-2} to 10^{-8} for the networks. For our matching data, we chose a cutoff of 10^{-3} for each network, because it offers a compromise between complex-cluster matching rate and a cluster passing rate, i.e., we can get a large sample of clusters with high matching rates (see Section 3.2).

2.5 Matching Criteria

We need to develop matching criteria to decide whether a given PPI network cluster matches a known biological complex. From the standpoint of considering the practicality of our results, it makes sense to consider a predicted cluster and a known protein complex to be matched if a

large proportion of each protein (node) set overlaps, or if the set of cluster nodes is nearly entirely contained within a set of proteins in a complex. Having a large cluster containing a small complex is not as useful in application to a lab setting, so we do not consider this case.

For a very large protein complex and a matching PPI network cluster, a given overlap proportion is more significant than it would be in a small complex and a matching cluster. For example, an overlap of five proteins between a complex and a cluster each of size six is less significant (i.e., more likely to occur at random) than an overlap of fifty proteins between a complex and a cluster each of size sixty. Bearing this in mind, we consider a cluster Cl to match a complex Co by overlap if both

$$\frac{|V(Cl) \cup V(Co)|}{|V(Cl)|} \geq \frac{P_{\text{cluster}}}{\log_{10}(7 + |V(Cl)|)} \quad (2)$$

and

$$\frac{|V(Cl) \cup V(Co)|}{|V(Co)|} \geq \frac{P_{\text{complex}}}{\log_{10}(7 + |V(Co)|)} \quad (3)$$

are satisfied, and we consider a cluster to match a complex by containment if

$$\frac{|V(Cl) \cup V(Co)|}{|V(Cl)|} \geq P_{\text{contain}}. \quad (4)$$

For these three equations, P_{cluster} , P_{complex} , and P_{contain} are all user-defined, experimentally derived proportions between 0 and 1. Note that in matching analysis, we do not consider proteins in a given protein complex if they do not appear in the applicable PPI network. Adding 7 to $|V(Cl)|$ in Equations 2 and 3 is done because it was empirically found to yield good thresholds. In fact, Equations 2 to 4 are entirely the result of empirical results: the equations generate sensible values such that a match is an overlap that represents high statistical significance without being too stringent a requirement (see Figure 1 and Section 3.2).

2.6 The RNSC Algorithm

A clustering of a network $G(V, E)$ is a decomposition of the set of nodes V into subsets of nodes that are highly interconnected (i.e., these subsets of nodes *induce* dense subgraphs). Our clustering algorithm is the

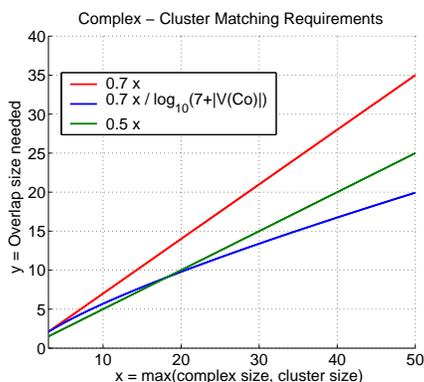


Figure 1: The overlap requirements for a match between a cluster and a complex. The x -axis is the larger of the complex size and the cluster size, and the y -axis is the overlap size needed to consider the complex and the cluster to be matched. The lines $y = 0.5x$ and $y = 0.7x$ are given for reference only.

Restricted Neighbourhood Search Clustering Algorithm (RNSC), which is a cost-based local search algorithm based loosely on the tabu search meta-heuristic (Glover, 1989). In the context of this algorithm, a clustering of a network $G = (V, E)$ is equivalent to a partitioning of the node set V . RNSC efficiently searches the space of partitionings of V , each of which is assigned a cost, for a clustering with low cost. The algorithm searches using a simple integer-valued cost function (called the *naive cost function*) as a preprocessor before it searches using a more expressive (but less efficient) real-valued cost function (called the *scaled cost function*). The initial clustering is random or user-input.

RNSC searches for a low-cost clustering by first composing an initial random clustering, then iteratively moving one node from one cluster to another in a randomized fashion to improve the clustering’s cost. A general move is one that lowers the clustering cost by a near-optimal amount. The common problem among local search algorithms is their tendency to settle in poor local minima. This problem can be largely avoided by using diversification and multiple experiments. Thus, our algorithm makes diversification moves, which shuffle the clustering by occasionally dispersing the contents of a cluster at random.

In addition, RNSC maintains a list of tabu (forbidden) moves to prevent cycling back to the previously explored partitioning. Since RNSC is randomized, different runs on the same input data will result in different output clusterings.

The algorithm maintains a multitude of data structures and incurs a large memory cost for the sake of time-efficiency. Ordinarily, maintenance of the data structures for such a search algorithm would present a prohibitive cost in computation. However, there are many problem-specific properties related to both graph clustering and the chosen cost functions that allow RNSC to perform very efficiently (a more detailed explanation of the RNSC algorithm can be found on the supplementary information web page (King *et al.*, 2004) and in (King, 2004)). Once RNSC outputs a clustering, we must filter the clusters so that we retain only those clusters which are very likely to correspond to true protein complexes. We do this by setting a maximum P -value for functional homogeneity, a minimum density, and a minimum size. The clusters which fall within these criteria are our predicted protein complexes.

3 Experiments and Results

Each network was clustered at least four times using the RNSC algorithm running under Linux. Each run took between ten seconds and three hours on a 2.8GHz processor, with Y_{2k} being the fastest and F_{20k} being the most time consuming. We then took the lowest-cost clustering produced by these runs for each network. These clusterings are available on the supplementary data page (King *et al.*, 2004).

The values that we chose for the matching thresholds are $P_{\text{cluster}} = P_{\text{complex}} = .7$ and $P_{\text{contain}} = .9$. The thresholds for cluster size, density, and functional homogeneity are a matter of compromise: although increasing the strictness of the thresholds generally increased the prediction rate, it also reduced the number of passing predictions (see Section 3.1). In the case where few protein complexes are known for the PPI network (e.g. fruitfly and worm), this scalability is extremely useful: we can make the thresholds very strict to begin with, and relax them as we analyze the growing set of predicted protein complexes (clusters).

Network	Minimum size	Total clusters	Passing clusters
Y_{2k}	4	393	48
Y_{11k}	5	974	84
Y_{45k}	7	1815	86
Y_{78k}	8	1811	90

Table 1: Cluster size lower bounds for *S. cerevisiae* PPI networks' clusters, needed to pass through the filter. For example, for Y_{2k} network, out of 393 clusters in total, 48 were of size at least 4.

3.1 Filter Cutoffs

All of the three filter cutoffs (for cluster size, density, and functional homogeneity) were chosen to yield reasonably high sample sizes while ensuring that clusters passing through the filter had a good chance of matching known complexes. In the case of the yeast networks, the minimum cluster size cutoff increased with the size of the network accordingly. Table 1 shows the chosen size cutoffs for the yeast PPI networks, along with the sizes of the cluster sets that pass the size cutoff.

We imposed a lower bound on the density of predicted complexes. As seen in Figure 2, a significant decrease in the passing rate of RNSC clusters occurs when the cluster density cutoff is between .65 and .75. In general, known complexes tend to have high density in the PPI network, but very few large complexes have density 1 (King *et al.*, 2004). A density cutoff in the range of .65 and .75 allows a good compromise between passing sample size and prediction rate, but a cutoff closer to .9 may give a very high passing rate in a small sample size. For experimental results in the yeast networks, we used a cutoff of .7.

As with cluster size and density, for functional homogeneity (P -value) filtering we wish to maintain both a reasonable sample size and a high matching rate among passing clusters. Figure 3 shows the effect of changing thresholds for both density and P -value (after filtering for size) in Y_{78k} . Figure 4 shows the effect of these thresholds on the sample size in Y_{78k} . For our experimental cluster passing rates, we chose a P -value cutoff of 10^{-3} .

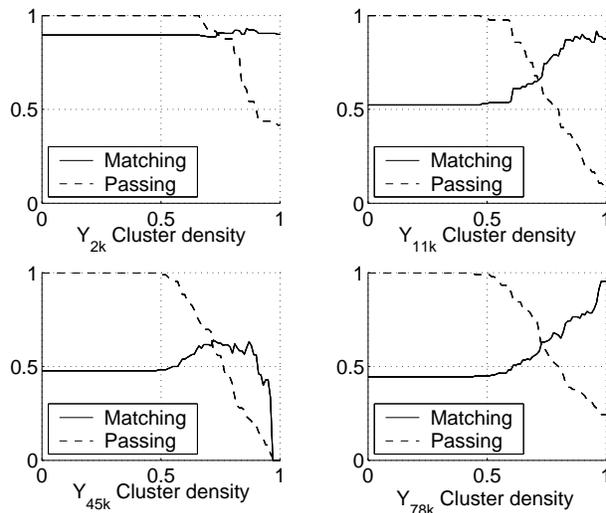


Figure 2: The proportion of RNSC clusters which pass the cluster density filter (i.e., cluster passing rate) and the proportion of these passing clusters that match known complexes (cluster matching rate) for yeast networks Y_{2k} , Y_{11k} , Y_{45k} , and Y_{78k} . These rates are for clusters that have already been filtered for size, but not for functional homogeneity.

3.2 Results

Matching rates for the yeast networks are shown in Table 2 for density $\geq .7$ and $P \leq 10^{-3}$, using the size cutoffs found to provide good passing sample sizes (described in Table 1). The fact that all of the filter cutoffs can be adjusted means that there are countless samples of varying size and matching rate. An example is presented in Figure 5, where each choice of filter cutoffs is represented as a point. In spite of the noise, the results for Y_{78k} are the best: for a given false positive rate, the true positive rate for Y_{78k} is the highest of the four. This may be because the larger data set carries much more statistical significance, in spite of it containing more noise.

Figure 6A shows an example of a predicted complex (i.e., a RNSC cluster) and the true protein complex from MIPS that it matches in the yeast network Y_{11k} . The RNSC cluster has size 8, density .964, and P -value 3.98×10^{-8} . The known cluster, COPI, has size 8, density

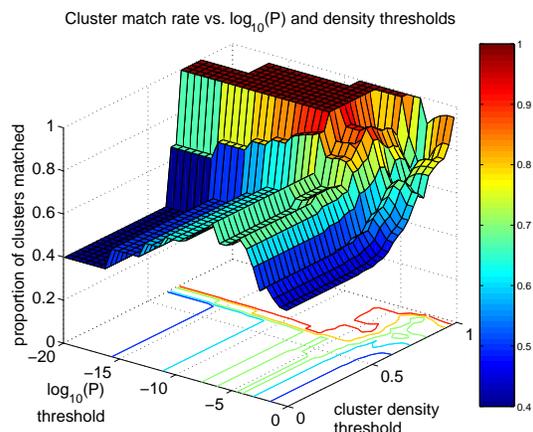


Figure 3: Proportion of passing clusters in Y_{78k} which match a known complex from MIPS. The sample is the set of clusters passing first the size restriction, then the P -value restriction and density restriction. The P -value and density restrictions are given on the x - and y -axes. We chose $.7$ and 10^{-3} as our density and P -value cutoffs, respectively.

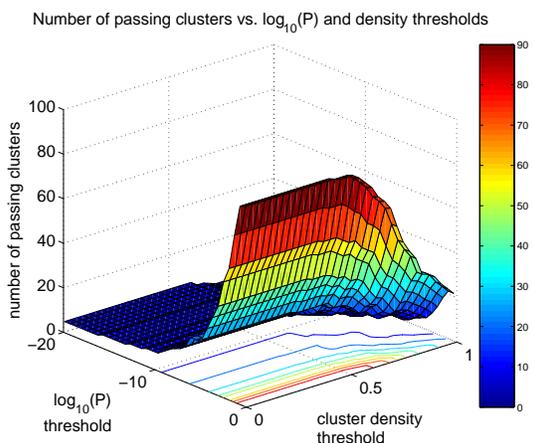


Figure 4: The effect of changing P -value and density cut-offs on the sample size, i.e. the number of clusters that pass the filter criteria for Y_{78k} . Clusters are first filtered by size, then by P -value and density. We chose $.7$ and 10^{-3} as our density and P -value cutoffs, respectively.

Graph	Minimum size	Passing clusters	Matched clusters	Prediction rate
Y_{2k}	4	28	23	82.1%
Y_{11k}	5	45	30	66.7%
Y_{45k}	7	32	21	65.6%
Y_{78k}	8	31	22	73.3%

Table 2: Matching data for density $\geq .7$ and $P \leq 10^{-3}$. Passing clusters are those that pass all filtering criteria, and matching clusters are those passing clusters that satisfy the matching criteria with at least one complex from MIPS.

.786, and P -value 3.29×10^{-10} . They share 7 proteins. COPI is a multimeric complex that contributes to the coating of membrane vesicles within the cell. Although the protein Arf1, which is in COPI but not the predicted complex, has the same gene ontological function as the rest of the proteins in COPI, it is incident with only one edge in the complex. Gpt2, which is contained in the predicted complex but not the known complex, is incident with six edges in the cluster. Gpt2 is glutamic pyruvate transaminase 2, and is responsible for transaminase and transferase activity. Although Gpt2 is assigned a different functional group by von Mering *et al.*, according to MIPS, each of these nine proteins is responsible for cellular transport, transport facilitation, and transport routes (Mewes *et al.*, 2002; von Mering *et al.*, 2002). This suggests that Gpt2 likely belongs to the COPI complex.

Figure 6B shows an unmatched RNSC cluster in Y_{11k} . In fact, this cluster has no more than one protein shared with any known complex. However, it exhibits all of the properties that we are looking for: it has sufficient size, 7, and high density, $.810$; its functional homogeneity P -value is 9.31×10^{-6} , with six of its seven proteins contributing to energy production. This suggests that biological validation of this set of proteins forming a protein complex may be worthwhile.

Figure 6C shows an RNSC cluster in Y_{11k} that is contained within a MIPS complex. This is a good example of a containment match; note that the cluster contains most of the edges within the complex. Indeed, the nodes of the complex that are not included in the cluster do not exhibit the ideal graph-theoretic properties of protein complexes at all. They are sparsely connected and largely heteroge-

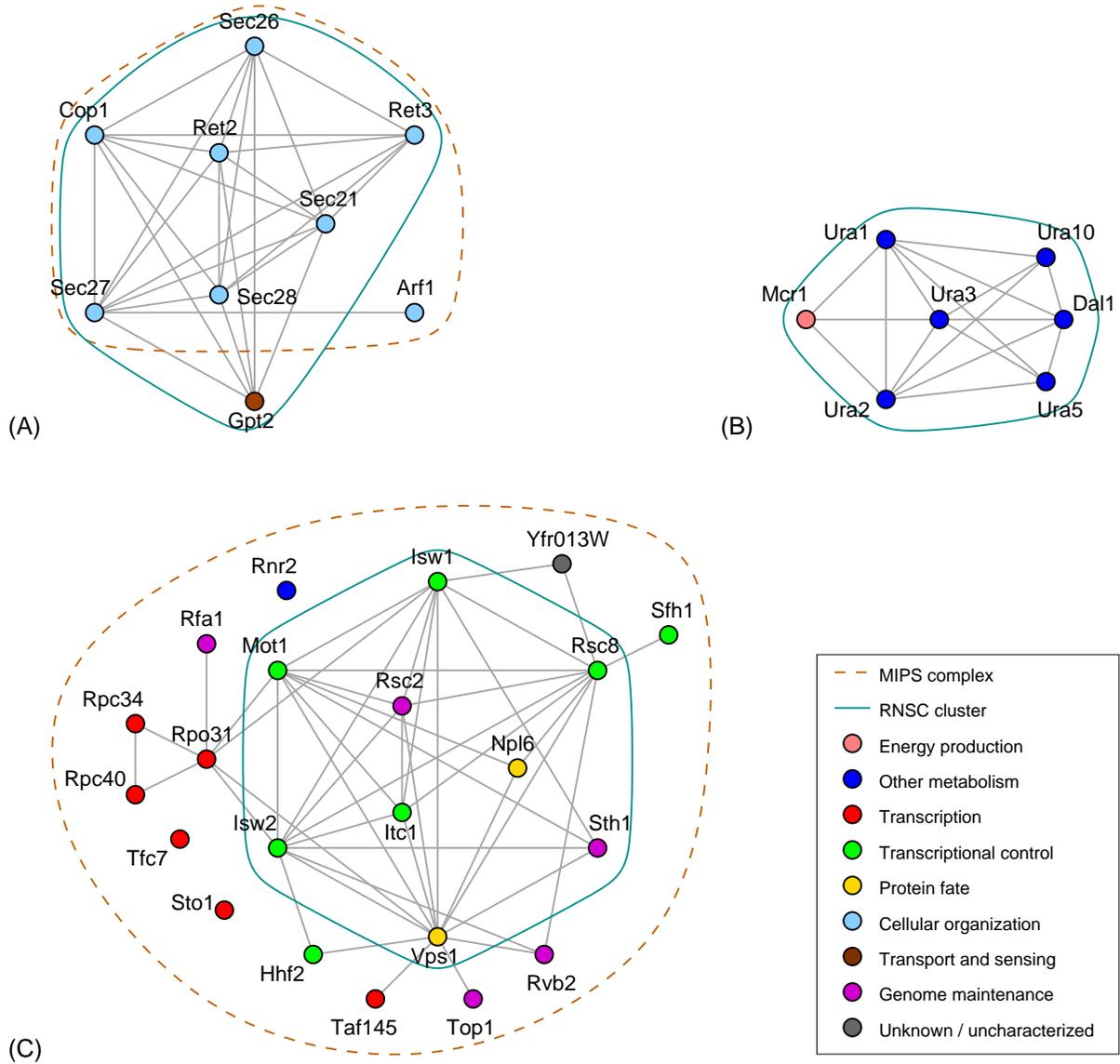


Figure 6: Examples of matched and predicted protein complexes: **(A)** MIPS complex COPI in the yeast network Y_{11k} and the matching complex predicted by RNSC. Each has size 8, and their overlap is 7. **(B)** An unmatched cluster in the yeast network Y_{11k} . The cluster has no overlap greater than one protein with any known complex. It passed through the filter, and exhibits characteristics of a protein complex. **(C)** This RNSC cluster in Y_{11k} is contained within a larger MIPS complex. Note that the cluster contains most of the edges in the complex.

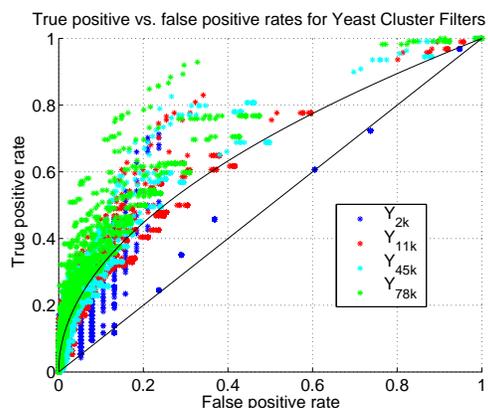


Figure 5: True positive rate vs. false positive rate for filtered yeast clusters: the proportion of matched clusters accepted by the filter vs. the proportion of unmatched clusters accepted by the filter. The points represent all tested filter cutoffs for size, P -value, and density. Clusters of size less than two are ignored in this data. The curve $y = \sqrt{x}$ is given as a reference.

neous. This MIPS complex is responsible for transcription, DNA maintenance, and chromatin structure.

As in (Pržulj *et al.*, 2004), Rib1-5, Rib7 is a functionally homogeneous cluster with density 1.0 in Y_{11k} . In Y_{2k} , however, only Rib1, Rib3, and Rib5 exist, and they make up a cluster of density .67. In Y_{45k} and Y_{78k} , Rib1-5 and Rib7 have density 1.0 among themselves, but the proteins are highly interactive with other proteins. The result is that Rib1-5, Rib7 is not a cluster in either of these two PPI networks, in spite of the fact that they likely represent a complex; rather, the Rib proteins are divided among several clusters. This is a case in which hierarchical cluster analysis may lend some insight, that is, considering all four networks for yeast simultaneously. None of these proteins is represented in the MIPS data, so there is no match to be considered.

The results for the fly and worm networks are less definitive. Because there are no comprehensive sources for complexes and functional classifications for these networks, we could neither construct P -values for the clusters nor compare them to a set of known complexes. In these networks, we filtered clusters for size and density.

The predicted complexes are given in the supplementary data (King *et al.*, 2004). For F_{20k} , there are only 5 predicted complexes, the largest of which has size 5. This is due to the fact that the current fruitfly network is extremely sparse. For F_{5k} , the less noisy data set for fruitfly, there are 42 predicted complexes, all of size 3 and 4. For W_{5k} , there are 32 predicted complexes, including 3 of size ≥ 10 . In the future, more complete PPI data will likely lead to a larger, more significant set of predicted complexes for fly and worm.

3.3 Discussion

Protein complexes have a number of inherent graph-theoretical and gene-ontological properties. As seen in the results, using size, density, and functional homogeneity as filtering criteria for network clusters is a reasonable way to predict protein complexes. However, there are some problems with the method. While protein complexes are usually expected to have high density in PPI networks, not all do. A related problem is the incompleteness of current PPI networks. The more complete and accurate our PPI and known protein complexes data sets are, the better we will be equipped to analyze the PPI networks.

Further, the functional homogeneity, while accurate for the most part, seems to be an incomplete, oversimplified model. Many known complexes show very low functional homogeneity. Also, many proteins belong to multiple functional groups. In addition, many proteins are of unknown function.

Even with such a simple filtering model and incomplete data, we managed to achieve very high matching rates between PPI network clusters and known protein complexes (Table 2). In comparison, Bader and Hogue generate a set of 209 predicted complexes, of which 54 match the MIPS database in at least 20% of their proteins in a yeast PPI network of some 15,000 interactions (Bader & Hogue, 2003). In (Pržulj *et al.*, 2004), a set of 31 predicted complexes is given for Y_{11k} , of which 27 were reported to have high overlaps with MIPS complexes. Jansen *et al.* predict pairs of nodes to be in the same cluster; they, like us, achieve low error rates (as low as 0% for 5 predicted pairs) that increase with the sample size. However, their findings cannot easily be applied to predicting entire complexes, but only interactions within them (Jansen *et al.*,

2002). Our results complement these efforts to better understand protein complexes within networks or protein-protein interactions.

4 Conclusions and Future Work

Using the RNSC algorithm to cluster PPI networks and filtering based on graph-theoretic resemblance to typical known protein complexes provides an effective method for predicting protein complexes. In general, it is becoming more and more evident that employing graph-theoretic techniques can be very useful in protein network analysis, as also demonstrated by recent research (Pržulj *et al.*, 2004; Pržulj, 2004; Yu *et al.*, 2004). Our results suggest that we can predict protein complexes with high confidence using RNSC algorithm with filtering. These predictions can be used to make wet lab experiments more focused, efficient, and inexpensive. Not only do the results warrant investigation where predicted complexes are unknown, but in some cases they warrant reexamination of current results. In order for this predictor (and other graph-theoretic tools) to work best, our knowledge of the networks needs to be improved. As more PPI data becomes available, automated tools for their analysis will need to become scalable and accurate..

There is a huge amount of further research to be done in the area of PPI network analysis. On the side of gene ontology, it will likely be helpful to investigate improved functional homogeneity models. Clearly, the mono-functional model of functional homogeneity that we use stands to be improved, most likely at a cost of simplicity. Just as protein function can be used to help predict protein complexes, knowledge of complexes can be used to predict previously unknown cellular function (Bu *et al.*, 2003).

On the side of clustering, it will likely be very instructive to investigate hierarchical complex predictions: In the case of yeast, for example, we predicted protein complexes using four PPI networks of increasing size. How do the predicted complexes in one such network relate to those in another network? Hopefully knowledge of this will give us further insight to the nature of protein interactions.

We have developed an accurate and scalable method of predicting protein complexes from PPI network data.

Cellular research will inevitably continue to be led by biological experiments, but computational analysis methods, such as ours, are likely to become indispensable for their ability to identify areas of significance at an extremely low cost.

5 Acknowledgements

This work was supported by the University of Toronto (AK), OGS (NP), NIH P50 GM-62413 (NP, IJ), the National Science and Engineering Research Council RGPIN 203833-02 (IJ). AK would like to thank Rudi Mathon for his guidance and support.

References

- Albert, R. & Barabási, A.-L. (2002) Statistical mechanics of complex networks. *Reviews of Modern Physics*, **74**, 47–97.
- Bader, G. & Hogue, C. (2003) An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics*, **4** (2).
- Barabási, A.-L. & Oltvai, Z. N. (2004) Network biology: understanding the cell's functional organization. *Nature Reviews Genetics*, **5**, 101–113.
- Bu, D., Zhao, Y., Cai, L., Xue, H., Zhu, X., Lu, H., Zhang, J., Sun, S., Ling, L., Zhang, N., Li, G. & Chen, R. (2003) Topological structure analysis of the protein-protein interaction network in budding yeast. *Nucleic Acids Research*, **31** (9), 2443–2450.
- Gavin, A. C., Bosche, M., Krause, R., Grandi, P., Marzioch, M. & Bauer, A. (2003) A functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature*, **415** (6868), 141–147.
- Giot, L., Bader, J. S., Brouwer, C., Chaudhuri, A., Kuang, B., Li, Y., Hao, Y. L., Ooi, C. E., Godwin, B., Vitols, E., Vijayadamodar, G., Pochart, P., Machineni, H., Welsh, M., Kong, Y., Zerhusen, B., Malcolm, R.,

- Varrone, Z., Collis, A., Minto, M., Burgess, S., McDaniel, L., Stimpson, E., Spriggs, F., Williams, J., Neurath, K., Ioime, N., Agee, M., Voss, E., Furtak, K., Renzulli, R., Aanensen, N., Carrola, S., Bickelhaupt, E., Lazovatsky, Y., DaSilva, A., Zhong, J., Stanyon, C. A., Finley, R. L., Jr., White, K. P., Braverman, M., Jarvie, T., Gold, S., Leach, M., Knight, J., Shimkets, R. A., McKenna, M. P., Chant, J. & Rothberg, J. M. (2003) A protein interaction map of *Drosophila melanogaster*. *Science*, **302** (5651), 1727–1736.
- Glover, F. (1989) Tabu search, part I. *ORSA Journal on Computing*, **1** (3), 190–206. “ORSA” is called Informs today.
- Hartuv, E. & Shamir, R. (2000) A clustering algorithm based on graph connectivity. *Information Processing Letters*, **76** (4–6), 175–181.
- Ho, Y., Gruhler, A., Heilbut, A., Bader, G. D., Moore, L., Adams, S. L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., Yang, L., Wolting, C., Donaldson, I., Schandorff, S., Shewnarane, J., Vo, M., Taggart, J., Goudreaault, M., Muskat, B., Alfarano, C., Dewar, D., Lin, Z., Michalickova, K., Willems, A. R., Sassi, H., Nielsen, P. A., Rasmussen, K. J., Andersen, J. R., Johansen, L. E., Hansen, L. H., Jespersen, H., Podtelejnikov, A., Nielsen, E., Crawford, J., Poulsen, V., Sorensen, B. D., Matthiesen, J., Hendrickson, R. C., Gleeson, F., Pawson, T., Moran, M. F., Durocher, D., Mann, M., Hogue, C. W., Figeys, D., & Tyers, M. (2003) Systematic identification of protein complexes in *saccharomyces cerevisiae* by mass spectrometry. *Nature*, **415** (6868), 180–183.
- Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M. & Sakaki, Y. (2001) A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Nat'l. Acad. Sci. USA*, **98** (8), 4569–4574.
- Ito, T., Tashiro, K., Muta, S., Ozawa, R., Chiba, T., Mishizawa, M., Yamamoto, K. & S. Kuhara, a. Y. S. (2000) Toward a protein-protein interaction map of the budding yeast: a comprehensive system to examine two-hybrid interactions in all possible combinations between the yeast proteins. *Proc. Nat'l. Acad. Sci. USA*, **97** (3), 1143–1147.
- Jansen, R., Lan, N., Qian, J. & Gerstein, M. (2002) Integration of genomic datasets to predict protein complexes in yeast. *J. Struct. Funct. Genomics*, **2**, 71–81.
- King, A., Pržulj, N. & Jurisica, I. (2004). Supplementary information for *protein complex prediction via cost-based clustering*. www.cs.utoronto.ca/~juris/data/ppi04.
- King, A. D. (2004). Graph clustering with restricted neighbourhood search. Master's thesis University of Toronto.
- Li, S., Armstrong, C. M., Bertin, N., Ge, H., Milstein, S., Boxem, M., Vidalain, P.-O., Han, J.-D. J., Chesneau, A., Hao, T., Goldberg, D. S., Li, N., Martinez, M., Rual, J.-F., Lamesch, P., Xu, L., Tewari, M., Wong, S. L., Zhang, L. V., Berriz, G. F., Jacotot, L., Vaglio, P., Reboul, J., Hirozane-Kishikawa, T., Li, Q., Gabel, H. W., Elewa, A., Baumgartner, B., Rose, D. J., Yu, H., Bosak, S., Sequerra, R., Fraser, A., Mango, S. E., Saxton, W. M., Strome, S., van den Heuvel, S., Piano, F., Vandenhaute, J., Sardet, C., Gerstein, M., Doucette-Stamm, L., Gunsalus, K. C., Harper, J. W., Cusick, M. E., Roth, F. P., Hill, D. E. & Vidal, M. (2004) A map of the interactome network of the metazoan *C. elegans*. *Science*, **303** (5657), 540–543.
- Mewes, H. W., Frishman, D., Guldener, U., Mannhaupt, G., Mayer, K., Mokrejs, M., Morgenstern, B., Munsterkotter, M., Rudd, S. & Weil, B. (2002) Mips: a database for genomes and protein sequences. *Nucleic Acids Research*, **30** (1), 31–34.
- Newman, M. E. J. (2003) The structure and function of complex networks. *SIAM Review*, **45** (2), 167–256.
- Pržulj, N. (2004) Graph theory approaches to protein interaction data analysis. In *Knowledge Discovery in High-Throughput Biological Domains*, (Jurisica, I. & Wigle, D., eds), Interpharm/CRC.
- Pržulj, N., Wigle, D. & Jurisica, I. (2004) Functional topology in a network of protein interactions. *Bioinformatics*, **20** (3), 340–348.

- Strogatz, S. H. (2001) Exploring complex networks. *Nature*, **410**, 268–276.
- Uetz, P., Giot, L., Cagney, G., Mansfield, T. A., Judson, R. S., Knight, J. R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleish, T., Vijayadamar, G., Yang, M., Johnston, M., Fields, S. & Rothberg, J. M. (2000) A comprehensive analysis of protein-protein interactions in *saccharomyces cerevisiae*. *Nature*, **403** (6770), 623–627.
- von Mering, C., Krause, R., Snel, B., Cornell, M., Oliver, S. G., Fields, S. & Bork, P. (2002) Comparative assessment of large-scale data sets of protein-protein interactions. *Nature*, **417** (6887), 399–403.
- West, D. B. (2001) *Introduction to Graph Theory, Second Edition*. Prentice Hall, Upper Saddle River, NJ.
- Yu, H., Zhu, X., Greenbaum, D., Karro, J. & Gerstein, M. (2004) Topnet: a tool for comparing biological subnetworks, correlating protein properties with topological statistics. *Nucleic Acids Research*, **32** (1), 328–337.