Uncovering Biological Network Function via Graphlet Degree Signatures

Tijana Milenković and Nataša Pržulj *

Department of Computer Science, University of California, Irvine, CA 92697-3435, USA

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ABSTRACT

Motivation: Proteins are essential macromolecules of life and thus understanding their function is of great importance. The number of functionally unclassified proteins is large even for simple and well studied organisms such as baker’s yeast. Methods for determining protein function have shifted their focus from targeting specific proteins based solely on sequence homology to analyses of the entire proteome based on protein-protein interaction (PPI) networks. Since proteins aggregate to perform a certain function, analyzing structural properties of PPI networks may provide useful clues about the biological function of individual proteins, protein complexes they participate in, and even larger subcellular machines.

Results: We design a sensitive graph theoretic method for comparing local structures of node neighborhoods that demonstrate that in PPI networks, biological function of a node and its local network structure are closely related. The method groups topologically similar proteins under this measure in a PPI network and shows that these protein groups belong to the same protein complexes, perform the same biological functions, are localized in the same subcellular compartments, and have the same tissue expressions. Moreover, we apply our technique on a proteome-scale network data and infer biological function of yet unclassified proteins demonstrating that our method can provide valuable guidelines for future experimental research.

Availability: Data is available upon request.

Contact: natasha@ics.uci.edu

1 INTRODUCTION

Large amounts of biological network data are becoming available. We study protein-protein interaction (PPI) networks (or graphs), in which nodes correspond to proteins and undirected edges represent physical interactions between them. Since a protein almost never acts in isolation, but rather interacts with other proteins in order to perform a certain function, PPI networks by definition reflect the interconnected nature of biological processes. Analyses of PPI networks may give valuable insight into biological mechanisms and provide deeper understanding of complex diseases. Defining the relationship between the PPI network topology and biological function and inferring protein function from it is one of the major challenges in the post-genomic era. [Nabieva et al., 2005; Vazquez et al., 2003; Schwikowski and Fields, 2000; Hishigaki et al., 2001; Letovsky and Kasif, 2003; Deng et al., 2003, 2004; Brun et al., 2004].

1.1 Background

Various approaches for determining protein function from PPI networks have been proposed. “Neighborhood-oriented” approaches observe the neighborhood of a protein to predict its function by finding the most common function(s) among its neighbors. The “majority rule” approach considers only nodes directly connected to the protein of interest [Schwikowski and Fields, 2000]. An improvement is made by also observing indirectly connected level-2 neighbors of a node [Chua et al., 2006]. Furthermore, the function with the highest $\chi^2$ value amongst the functions of all “n-neighboring proteins” is assigned to the protein of interest [Hishigaki et al., 2001]. Other approaches use the idea of shared neighbors [Samanta and Liang, 2003] or the network flow-based idea [Nabieva et al., 2005] to determine protein function.

Several global optimization-based function prediction strategies have also been proposed. Any given assignment of functions to the whole set of unclassified proteins in a network is given a score, counting the number of interacting pairs of nodes with no common function; the functional assignment with the lowest score maximizes the presence of the same function among interacting proteins [Vazquez et al., 2003]. An approach that reduces the computation requirements of this method has been proposed [Sun et al., 2006].

Cluster-based approaches are exploiting the existence of regions in PPI networks that contain a large number of connections between the constituent proteins. These dense regions are a sign of the common involvement of those proteins in certain biological processes. The restricted-neighborhood-search clustering algorithm efficiently partitions a PPI network into clusters identifying known and predicting unknown protein complexes [King et al., 2004]. Similarly, highly connected subgraphs are used to identify clusters in networks [Hartuv and Shamir, 2000], defining the relationship between the PPI network size and the number and complexity of the identified clusters, and identifying known protein complexes from these clusters [Pržulj et al., 2004]. Moreover, Czekanowski-Dice distance is used for protein function prediction by forming clusters of proteins sharing a high percentage of interactions [Brun et al., 2004].
1.2 Approach

We address the above mentioned challenge. First, we verify that in PPI networks of yeast and human, local network structure and biological function are closely related. We do this by designing a method that clusters together nodes of a PPI network with similar topological surroundings and by demonstrating that it successfully uncovers groups of proteins belonging to the same protein complexes, performing the same biological functions, being localized in the same subcellular compartments, and having the same tissue expressions. Since we verify this for PPI networks of a unicellular and a multicellular eukaryotic organism (yeast and human, respectively), we hypothesize that PPI network structure and biological function are related in other eukaryotic organisms as well. Next, since the number of functionally unclassified proteins is large even for simple and well studied organisms such as baker’s yeast *Saccharomyces cerevisiae* (Peña-Castillo and Hughes, 2007), we describe how to apply our technique to predict membership in protein complexes, functional groups, and subcellular compartments of yet unclassified yeast proteins.

Our method belongs to the group of clustering-based approaches. However, compared to other methods that define a cluster as a dense interconnected region of a network, our method defines it as a set of nodes with similar topological *signatures* (defined below). Thus, nodes belonging to the same cluster do not need to be connected or belong to the same part of the network.

2 METHODS

Our new measure of node similarity generalizes the degree of a node, which counts the number of edges that the node touches, into the vector of *graphlet degrees*, counting the number of graphlets that the node touches; graphlets are small connected non-isomorphic induced subgraphs of a large network (Pržulj et al., 2004) (see Figure 1). As opposed to partial subgraphs (e.g., network *motifs* (Milo et al., 2002)), graphlets must be induced, i.e., they must contain all edges between the nodes of the subgraph that are present in the large network. We count the number of graphlets touching a node for all 2-5-node graphlets, denoted by \(G_0, G_1, \ldots , G_{29}\) in Figure 1; counts involving larger graphlets become computationally infeasible for large networks.

Clearly, the degree of a node is the first one in this vector, since an edge (graphlet \(G_{0}\)) is the only 2-node graphlet. We call this vector the *signature* of a node. It is topologically relevant to distinguish between nodes touching a 3-node linear path (graphlet \(G_1\)) at an end, or at the middle node; we provide a mathematical formulation of this phenomenon for all graphlets with 2-5 nodes. This is summarized by *automorphism orbits* (or just orbits, for brevity): by taking into account the “symmetries” between nodes of a graphlet, there are 72 different orbits for 2-5-node graphlets, numbered from 0 to 72 in Figure 1 (see Pržulj, 2006 for details). Thus, the signature vector of a node has 73 coordinates.

We compute node signature similarities as follows. We define a 73-dimensional vector \(W^i\) containing the weights \(w_i\) corresponding to orbits \(i \in \{0, \ldots , 72\}\), where weights are determined as follows. For each orbit, we consider the number of orbits affecting it. For example, the differences in orbit 0 (i.e., in the degree) of two nodes will automatically imply the differences in all other orbits, since all orbits depend on it. Each orbit \(i\) is assigned an integer \(o_i\) that represents the number of orbits that affect it (available upon request).

We consider that each orbit affects itself. We compute \(w_i\) as a function of \(o_i\). We need to assign a higher weight \(w_i\) to the orbits that are not affected by many other orbits. Thus, we apply a slow-increasing logarithm function to \(o_i\). Also, since the maximum value that an \(o_i\) can take is 73 (for 2-5-node graphlets), we divide \(\log_{10}(o_i)\) by \(\log_{10}(73)\) to scale it to \([0, 1]\). Since an orbit dependency count \(o_i\) of 1 indicates that no other orbits affect orbit \(i\) (i.e., this orbit is of the highest importance), we invert this scaled value of orbit dependencies as

\[
  w_i = 1 - \frac{\log_{10}(o_i)}{\log_{10}(73)}
\]

to assign the highest weight of 1 to orbit \(i\) with \(o_i = 1\). Clearly, \(w_i \in [0, 1]\) for all \(i \in \{0, \ldots , 72\}\) and orbits become less important as their weights \(w_i\) decrease.

For a node \(u\), we denote by \(u_i\) the \(i^{th}\) coordinate of its signature vector, i.e., \(u_i\) is the number of times node \(u\) touches orbit \(i\). We define the distance \(D_i(u, v)\) between the \(i^{th}\) orbits of nodes \(u\) and \(v\) as

\[
  D_i(u, v) = w_i \times \frac{[\log_2(u_i + 1) - \log_2(v_i + 1)]}{\log_2(\max\{u_i, v_i\} + 2)}
\]

We use \(\log_2\) in the numerator because the \(i^{th}\) coordinates of signature vectors of two nodes can differ by several orders of magnitude and we do not want the distance measure to be entirely dominated by these large values. Also, by using these logarithms, we take into account the relative difference between \(u_i\) and \(v_i\) instead of the absolute difference. We add 1 to \(u_i\) and \(v_i\) in the numerator of the formula for \(D_i(u, v)\) to prevent the logarithm function to go to infinity. We scale \(D_i\) to be in \([0, 1]\) by dividing with the value of the denominator in the formula for \(D_i(u, v)\). We add 2 in the denominator of the formula for \(D_i(u, v)\) to prevent it from being infinite or 0. We find the total distance \(D(u, v)\) between nodes \(u\) and \(v\) as

\[
  D(u, v) = \sum_{i=0}^{72} D_i(u, v) w_i
\]

Clearly, the distance \(D(u, v)\) is in \([0, 1]\), where distance 0 means the identity of signatures of nodes \(u\) and \(v\). Finally, the signature similarity, \(S(u, v)\), between nodes \(u\) and \(v\) is:

\[
  S(u, v) = 1 - D(u, v)
\]

For a node of interest, we form a cluster containing that node and all nodes in a network that are similar to it. According to the signature similarity metric, nodes \(u\) and \(v\) will be in the same cluster if their signature similarity \(S(u, v)\) is above a chosen threshold. We choose an experimentally determined threshold of 0.9-0.95. For thresholds above these values, only a few small clusters are obtained, especially for smaller PPI networks, indicating
too high stringency in signature similarities. For thresholds below 0.9, the clusters are very large, especially for larger PPI networks, indicating a loss of signature similarity. To illustrate signature similarities and our choices of signature similarity thresholds, in Figure 2 we present the signature vectors of yeast proteins in the PPI network of (Krogan et al., 2006) with signature similarity in Figure 2A and below 0.40 (Figure 2B). Signature vectors of proteins with high signature similarities follow the same pattern, while those of proteins with low signature similarities have very different patterns.

3 RESULTS AND DISCUSSION

We apply our method to six S. cerevisiae PPI networks and three human PPI networks. The S. cerevisiae PPI networks are henceforth denoted by “vonMering-core” (von Mering et al., 2002), “vonMering” (von Mering et al., 2002), “Krogan” (Krogan et al., 2004), “DIP-core” (Deane et al., 2002), “DIP” (Xenarios et al., 2002), and “MIPS” (Mewes et al., 2002). “vonMering-core” contains only high-confidence interactions described by von Mering et al. (von Mering et al., 2002); it contains 2,455 interactions amongst 988 proteins obtained mainly by tandem affinity purification (TAP) (Rigaut et al., 1999; Gavin et al., 2002); and High-Throughput Mass Spectrometric Protein Complex Identification (HMS-PCI) (Ho et al., 2004). “vonMering” is the PPI network containing the top 11,000 high-, medium-, and low-confidence interactions amongst 2,401 proteins described by von Mering et al. (von Mering et al., 2002); the dominant techniques used to identify PPIs in this network are TAP, HMS-PCI, gene neighborhood, and yeast-two-hybrid (Y2H). “Krogan” is the “core” PPI data set containing 7,123 interactions amongst 2,708 proteins obtained by TAP experiments as described by Krogan et al. (Krogan et al., 2006), “DIP-core” is the more reliable subset of the yeast PPI network from DIP (Xenarios et al., 2002) as described by Deane et al. (Deane et al., 2002); it contains 5,174 interactions amongst 2,210 proteins. “DIP” and “MIPS” are the yeast PPI networks downloaded in November 2007 from DIP (Xenarios et al., 2002) and MIPS (Mewes et al., 2002) databases, respectively; they contain 17,201 and 12,525 interactions amongst 4,932 and 4,786 proteins, respectively. The three human PPI networks that we analyze are henceforth denoted by “BIOGRID” (Stark et al., 2006), “HPRD” (Peri et al., 2004), and “Rual” (Rual et al., 2005). “BIOGRID” and “HPRD” are the human PPI networks downloaded in November 2007 from “BIOGRID” (Stark et al., 2006) and “HPRD” (Peri et al., 2004) databases, respectively; they contain 23,555 and 34,119 interactions amongst 7,941 and 9,182 proteins, respectively. “Rual” is the human PPI network containing 3,463 interactions amongst 1,873 proteins, as described by Rual et al. (Rual et al., 2005). We removed all self-loops and multiple edges from each of the PPI networks that we analyzed.

The entire PPI network is taken into account when computing signature similarities between pairs of nodes (i.e., proteins) and forming clusters (see section 2). However, here we only report the results of analyzing proteins involved in more than four interactions. We discard poorly connected proteins from our clusters because they are more likely to be involved in noisy interactions. Similar was done by Brun et al. (Brun et al., 2004). Note that the highest node degree in the analyzed PPI networks is 286. Also, we discard very small clusters containing less than three proteins. For the remaining clusters, we search for common protein properties: in yeast PPI networks, we look for the common protein complexes, functional groups, and subcellular localizations (described in MIPS (Mewes et al., 2002)) of proteins belonging to the same cluster; in human PPI networks, we look for the common biological processes, cellular components, and tissue expressions (described in HPRD (Peri et al., 2004)) of proteins in the same cluster.

Classification schemes and the data for the three protein properties that we analyzed in yeast PPI networks were downloaded from MIPS database (Mewes et al., 2002) in November 2007. For each of these three classification schemes (corresponding to protein complexes, biological functions, and subcellular localizations), we define two levels of strictness: the strict scheme uses the most specific MIPS annotations, and the flexible one uses the least specific ones. For example, for a protein complex “category” annotated by 510.190.900 in MIPS, the strict scheme returns 510.190.900, and the flexible one returns 510. Classification schemes and the data for the three protein properties that we analyzed in human PPI networks (corresponding to biological processes, cellular components, and tissue expressions) were downloaded from HPRD database (Peri et al., 2004) in November 2007. In order to test if our method clusters together proteins having the same protein properties, we refine our clusters by removing the nodes that are not contained in any of the yeast MIPS protein complex, biological function, or subcellular localization categories, or in any of the human HPRD biological process, cellular component, or tissue expression categories, respectively.

In our clusters, we measure the size of the largest common category for a given protein property as the percentage of the cluster size; we refer to it as the hit-rate. Clearly, a yeast protein can belong to more than one protein complex, be involved in more than one biological function, or belong to more than one subcellular compartment (and similar holds for human proteins). Thus, it is possible to have an overlap between categories, as well as more than one largest category in a cluster for a given protein property. We illustrate this for biological functions in the cluster presented in Figure 3, consisting of yeast proteins RPO26, SMD1, and SMB1. According to the strict scheme, protein SMD1 is in the common biological function category with protein RPO26 (16.03), as well as with protein SMB1 (11.04.03.01). Thus, there are two largest common biological function categories. The size of the largest common biological function category in the cluster is two and the hit-rate is $2/3=67\%$. For the flexible scheme, all three proteins are in one common biological function category (11) and thus, the size of the largest common biological function category is three and the hit-rate is $3/3=100\%$.

We also define the miss-rate as the percentage of the nodes in a cluster that are not in any common category with other nodes in the cluster, for a given protein property. For example, in Figure 4.
according to the strict scheme, proteins RPO26 and SMB1 are in a common biological function category with SMD1, but they themselves are not in any common biological function category. Although not all three proteins are in the same biological function category and the hit-rate is only 67%, the miss-rate is 0/3=0%, since every node is in at least one common biological function category with another node in the cluster. Clearly, the miss-rate for the flexible scheme is also 0/3=0%, since the three proteins are in the same biological function category (11) with respect to this scheme. Thus, if a protein belongs to several different categories for a given protein property (which is expected), the hit-rate in the cluster might be lower than 100% (as illustrated in Figure 5). Therefore, miss-rates are additional indicators of the accuracy of our approach.

For each of the six yeast PPI networks, the three yeast protein properties, and the two schemes, we measure the number of clusters (out of the total number of clusters in a network) having given hit- and miss-rates. We bin the hit- and miss-rates in increments of 10%. The results for the flexible scheme are presented in Figure 4. For subcellular localizations, in vonMering-core network, 86% of the clusters have hit-rate above 90%; for the remaining five networks, 65% of clusters have hit-rates above 60% (Figure 4A). For all networks, miss-rates for 72% of clusters are below 10% (Figure 4B). Similarly, for biological functions, the miss-rates in all six networks are under 10% for 81% of the clusters (Figure 4D). The hit-rates for biological functions are above 60% for 79% of the clusters in both von Mering networks; in the remaining four networks, 57% of the clusters have hit-rates above 50% (Figure 4C). Finally, for protein complexes, 47% clusters in vonMering-core, vonMering, and DIP-core networks have hit-rates above 60%, 36% of clusters in Krogan and MIPS networks have hit-rates above 50%, and 30% of clusters in DIP network have hit-rates above 40% (Figure 4E). Miss-rates for protein complexes are below 10% for 39% of the clusters in both von Mering networks and in DIP-core network; in the remaining three networks, 33% of the clusters have miss-rates below 39% (Figure 4F).

Similarly, for each of the three human PPI networks and their three protein properties that we analyzed, we measure the number of clusters (out of the total number of clusters in a network) having given hit- and miss-rates. The results are presented in Figure 5. For cellular components, in all three human PPI networks, 86% of the clusters have hit-rates above 50% (Figure 5A). Miss-rates for 68% of clusters in BIOGRID and HPRD networks are below 10%, while in Rual network 76% of clusters have miss-rates below 29% (Figure 5B). Similarly, for tissue expressions, hit-rates are above 50% for 74% of clusters in BIOGRID and HPRD networks, and for 98% of clusters in Rual network, respectively (Figure 5C). Miss-rates are lower than 10% for 61% of clusters in BIOGRID and HPRD networks, and for 48% of clusters in Rual network, respectively (Figure 5D). Finally, for biological processes, hit-rates are above 50% for 55% of clusters in BIOGRID network, for 45% of clusters in HPRD network, and for 33% of clusters in Rual network, respectively. (Figure 5E). Miss-rates are below 29% for 58% of the clusters in BIOGRID network and for 71% of the clusters in HPRD network; in Rual network, 44% of the clusters have miss-rates below 39% (Figure 5F).

To evaluate the effect of noise in PPI networks to the accuracy of our method, we compare the results for the high-confidence vonMering-core network and the lower-confidence vonMering network (Figure 4). As expected, clusters in the more noisy network have lower hit-rates compared to the high-confidence network. However, low miss-rates are still preserved in clusters of both networks for all three protein properties, indicating the robustness of our method to noise present in PPI networks.

Thus far, we demonstrated that our method identifies groups of nodes in PPI networks having common protein properties. Our technique can also be applied to predict protein properties of yet unclassified proteins by forming a cluster of proteins that are similar to the unclassified protein of interest and assigning it the most common properties of the classified proteins in the cluster. We do this for all 115 functionally unclassified yeast proteins from MIPS that have degrees higher than four in any of the six yeast PPI networks that we analyzed. In Tables 4 and 5, we present the predicted functions for proteins with prediction hit-rates of 50% or higher according to the strict and the flexible scheme, respectively. The full data set with functional prediction hit-rates lower than 50% is available upon request. Note that a yeast protein can belong to more than one yeast PPI network that we analyzed. Thus, biological functions that such proteins perform can be predicted from clusters
Fig. 4. The results of applying our method to the six yeast PPI networks (vonMering-core, vonMering, Krogan, DIP-core, DIP, and MIPS) and the three protein properties (subcellular localizations, biological functions, and protein complexes) in accordance with the flexible scheme: (A) hit-rates for subcellular localizations; (B) miss-rates for subcellular localizations; (C) hit-rates for biological functions; (D) miss-rates for biological functions; (E) hit-rates for protein complexes; (F) miss-rates for protein complexes.

derived from different yeast PPI networks. We observed an overlap of the predicted protein functions obtained from multiple PPI networks for the same organism, additionally verifying the correctness of our method. Furthermore, there exists overlap between our protein function predictions and those of others (Vazquez et al., 2003). Finally, we successfully predict the functional category of PWP1 protein that is still functionally uncharacterized in MIPS, but is characterized in SGD (Cherry et al., 1998) as being involved in rRNA processing.

To our knowledge, this is the first study that relates the PPI network structure to all of the following: protein complexes, biological functions, and subcellular localizations for yeast, and cellular components, tissue expressions, and biological processes for human.
Starting with the topology of PPI networks of different organisms that are of different sizes and are originating from a wide spectrum of small-scale and high-throughput PPI detection techniques, our method identifies clusters of nodes sharing common protein properties. Our method accurately uncovers groups of nodes belonging to the same protein complexes in the vonMering-core network: 44% of clusters have 100% hit-rate according to the flexible scheme. This additionally validates our method, since PPIs in this network are obtained mainly by TAP (Rigaut et al., 1999; Gavin et al., 2002) and HMS-PCI (Ho et al., 2002), which are known to favor protein complexes.

Our node similarity measure is highly constraining, since we take into account not only a node’s degree, but also additional 72 “graphlet degrees” (see section 2). Since the number of graphlets on n nodes increases exponentially with n, we use 2-5-node graphlets (see Figure 1). However, our method is easily extendible to include larger graphlets, but this would increase the computational complexity; the complexity is currently $O(|V|^5)$ for a graph $G(V, E)$, since we search for graphlets with up to 5 nodes. Nonetheless, since our algorithm is “embarrassingly parallel” (i.e., can easily be distributed over a cluster of machines), extending it to larger graphlets is feasible. In addition to the design of the signature similarity measure as a number in [0, 1], this makes our technique usable for larger networks.
Table 1. Predicted functions with prediction hit-rates of 50% or higher according to the strict scheme for yeast proteins that are unannotated in MIPS and that have degrees higher than four in any of the six yeast PPI networks. The column denoted by “Protein of interest” contains a protein of interest for which the function is predicted. The column denoted by “Degree” contains the degree of a given protein in the corresponding PPI network. The column denoted by “PPI Network” contains the PPI network from which the predicted function was derived. The column denoted by “Number of proteins in cluster” contains the total number of proteins in the cluster, including the protein of interest. The column denoted by “Number of unclassified proteins in cluster” contains the number of functionally unclassified proteins in a given cluster, including the protein of interest. The column denoted by “Majority (and predicted) function” contains the common functions amongst at least 50% proteins in the cluster that are also predicted functions for the protein of interest. The column denoted by “Number of proteins in cluster with the majority function” contains the number of nodes in the cluster with the majority function. The column denoted by “Hit-rate” contains the percentage of the total number of proteins in the cluster with the majority function; only the maximum hit-rate is reported for a protein of interest. Finally, the column denoted by “Miss-rate” contains the percentage of annotated nodes in the cluster that do not have a common function with any other annotated node in the cluster.

4 CONCLUSION

We present a new graph theoretic method for detecting the relationship between local topology and function in real-world networks. We apply it to proteome-scale PPI networks and demonstrate the link between the topology of a protein’s neighborhood in the network and its membership in protein complexes, functional groups, and subcellular compartments for yeast, and in cellular components, tissue expressions, and biological processes for human. Additionally, we demonstrate that our method can be used to predict biological function of uncharacterized proteins. Moreover, the method can be applied to different types of biological and other real-world networks and give insight into complex biological mechanisms and guidelines for future research.

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REFERENCES

Brun, C., Herrmann, C., and Guénoche, A. (2004). Clustering proteins from interaction networks for the prediction of cellular functions. *BMC Bioinformatics* 5.

Cherry, J. et al. (1998). SGD: Saccharomyces Genome Database. *Nucleic Acids Research* 26, 73–79.

Chua, H., Sung, W., and Wong, L. (2006). Exploiting indirect neighbours and topological weight to predict protein function from protein-protein interactions. *Bioinformatics* 22, 1623–1630.

Deane, C., Salwinski, L., Xenarios, I., and Eisenberg, D. (2002). Protein interactions: two methods for assessment of the reliability of high throughput observations. *Molecular and Cellular Proteomics* 1, 349–356.

Deng, M., Tu, Z., Sun, F., and Chen, T. (2004). Mapping gene ontology to proteins based on protein-protein interaction data. *Bioinformatics* 20, 895–902.

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REFERENCES

Brun, C., Herrmann, C., and Guénoche, A. (2004). Clustering proteins from interaction networks for the prediction of cellular functions. *BMC Bioinformatics* 5.

Cherry, J. et al. (1998). SGD: Saccharomyces Genome Database. *Nucleic Acids Research* 26, 73–79.

Chua, H., Sung, W., and Wong, L. (2006). Exploiting indirect neighbours and topological weight to predict protein function from protein-protein interactions. *Bioinformatics* 22, 1623–1630.

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| Protein of interest | Degree | PPI Network | Number of proteins in cluster | Number of unclassified proteins in cluster | Majority (and predicted) function | Number of proteins in cluster with the majority function | Hit-rate | Miss-rate |
|---------------------|--------|-------------|------------------------------|--------------------------------------------|----------------------------------|-----------------------------------------------|---------|-----------|
| PRPT (YLR306W)      | 22     | von Meir   | 23                           | 1                                         | TRANSCRIPTION                     | 17                                            | 77.22%  | 0.00%     |
| STOT (YLR352W)      | 42     | von Meir   | 6                            | 1                                         | PROTEIN WITH BINDING FUNCTION OR COFACTOR REQUIREMENT (putative catalytic) | 5                                             | 100.00% | 0.00%     |
| OCA1 (YMR022C)      | 10     | von Meir   | 3                            | 1                                         | CELL RESCUE, DEFENSE AND VIRULENCE | 2                                             | 100.00% | 0.00%     |
| YMR017C             | 8      | von Meir   | 3                            | 1                                         | PROTEIN SYNTHESIS                 | 2                                             | 100.00% | 0.00%     |
| YMR018C             | 7      | von Meir   | 3                            | 1                                         | PROTEIN SYNTHESIS                 | 2                                             | 100.00% | 0.00%     |
| YMR020C             | 6      | von Meir   | 3                            | 1                                         | PROTEIN FATE (putative, modification, destination) | 4                                             | 100.00% | 0.00%     |
| YMR022C             | 15     | von Meir   | 3                            | 1                                         | METABOLISM                        | 2                                             | 100.00% | 0.00%     |
| CC05 (YMR022C)      | 6      | Dip-core   | 9                            | 1                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 6                                             | 75.00%  | 12.50%    |
| YAD12W              | 19     | Krogan     | 9                            | 3                                         | TRANSCRIPTION                     | 4                                             | 66.67%  | 0.00%     |
| CC01 (YMR015W)      | 6      | Krogan     | 3                            | 1                                         | TRANSCRIPTION                     | 2                                             | 100.00% | 0.00%     |
| YDA08W              | 19     | Krogan     | 7                            | 2                                         | PROTEIN WITH BINDING FUNCTION OR COFACTOR REQUIREMENT (putative catalytic) | 4                                             | 80.00%  | 0.00%     |
| PBY1 (YMR034W)      | 23     | MIPS       | 6                            | 1                                         | CELL CYCLE AND DNA PROCESSING     | 3                                             | 66.67%  | 0.00%     |
| SHLK (YMR083C)      | 5      | MIPS       | 4                            | 1                                         | PROTEIN FATE (putative, modification, destination) | 2                                             | 66.67%  | 0.00%     |
| YER06W              | 5      | MIPS       | 7                            | 2                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 4                                             | 80.00%  | 20.00%    |
| YPT35 (YMR010W)     | 5      | MIPS       | 7                            | 2                                         | PROTEIN FATE (putative, modification, destination) | 3                                             | 66.67%  | 0.00%     |
| CH10 (YNR021C)      | 16     | MIPS       | 13                           | 2                                         | TRANSCRIPTION                     | 6                                             | 54.55%  | 9.09%     |
| LMI1 (YNR116C)      | 11     | MIPS       | 8                            | 2                                         | CELL CYCLE AND DNA PROCESSING     | 4                                             | 66.67%  | 0.00%     |
| YAR06W              | 7      | MIPS       | 4                            | 1                                         | METABOLISM                        | 2                                             | 66.67%  | 33.33%    |
| RAR61 (YNL010C)     | 5      | MIPS       | 9                            | 2                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 4                                             | 57.14%  | 14.29%    |
| EBS2 (YNR128W)      | 7      | MIPS       | 5                            | 1                                         | CELL CYCLE AND DNA PROCESSING     | 3                                             | 75.00%  | 0.00%     |
| SQ12 (YMR028W)      | 5      | MIPS       | 10                           | 1                                         | TRANSCRIPTION                     | 5                                             | 55.56%  | 0.00%     |
| YAR06W              | 6      | MIPS       | 4                            | 2                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 2                                             | 100.00% | 0.00%     |
| YAL010C             | 9      | Dip        | 3                            | 1                                         | PROTEIN FATE (putative, modification, destination) | 2                                             | 100.00% | 0.00%     |
| UPF3 (YLR077W)      | 38     | Dip        | 5                            | 2                                         | INTERACTION WITH THE ENVIRONMENT   | 2                                             | 66.67%  | 33.33%    |
| YAR028W             | 11     | Dip        | 3                            | 1                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 2                                             | 100.00% | 0.00%     |
| OCA1 (YMR022C)      | 8      | Dip        | 9                            | 2                                         | PROTEIN WITH BINDING FUNCTION OR COFACTOR REQUIREMENT (putative catalytic) | 6                                             | 86.67%  | 0.00%     |
| YER06W              | 8      | Dip        | 4                            | 2                                         | PROTEIN FATE (putative, modification, destination) | 2                                             | 100.00% | 0.00%     |
| YER06W              | 5      | Dip        | 6                            | 3                                         | CELL CYCLE AND DNA PROCESSING     | 2                                             | 66.67%  | 0.00%     |
| CC06 (YMR022C)      | 22     | Dip        | 5                            | 1                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 3                                             | 75.00%  | 25.00%    |
| YRF10C              | 5      | Dip        | 5                            | 1                                         | CELL CYCLE AND DNA PROCESSING     | 3                                             | 75.00%  | 0.00%     |
| YMR120W             | 61     | Dip        | 16                           | 2                                         | METABOLISM                        | 10                                            | 71.43%  | 7.14%     |
| YET1 (YLR024C)      | 51     | Dip        | 34                           | 4                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 16                                            | 50.00%  | 0.00%     |
| YLL020C             | 22     | Dip        | 17                           | 2                                         | CELLULAR TRANSPORT, TRANSPORT FACILITIES AND TRANSPORT ROUTES | 10                                            | 66.67%  | 0.00%     |
| RAR33 (YNL010C)     | 5      | Dip        | 9                            | 3                                         | METABOLISM                        | 2                                             | 66.67%  | 0.00%     |
| YMR017W             | 12     | Dip        | 4                            | 1                                         | METABOLISM                        | 2                                             | 66.67%  | 33.33%    |
| YMR018C             | 6      | Dip        | 6                            | 2                                         | TRINARY INTERACTION WITH THE ENVIRONMENT | 2                                             | 75.00%  | 0.00%     |
| YMR020W             | 6      | Dip        | 5                            | 1                                         | PROTEIN WITH BINDING FUNCTION OR COFACTOR REQUIREMENT (putative catalytic) | 3                                             | 100.00% | 0.00%     |

Table 2. Predicted functions with prediction hit-rates higher than 50% according to the flexible scheme for yeast proteins that are unannotated in MIPS and that have degrees higher than four in any of the six yeast PPI networks. The columns have the same meaning as in Table [1].
Deng, M., Zhang, K., Mehta, S., Chen, T., and Sun, F. (2003). Prediction of protein function using protein-protein interaction data. *Journal of Computational Biology* 10, 947–960.

Gavin, A. C. et al. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* 415(6868), 141–7.

Hartuv, E. and Shamir, R. (2000). A clustering algorithm based on graph connectivity. *Information Processing Letters* 76(4-6), 175–181.

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

Ho, Y. et al. (2002). Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry. *Nature* 415(6868), 180–183.

King, A. D., Pržulj, N., and Jurisica, I. (2004). Protein complex prediction via cost-based clustering. *Bioinformatics* 20(17), 3013–3020.

Krogan, N. et al. (2006). Global landscape of protein complexes in the yeast Saccharomyces cerevisiae. *Nature* 440, 637–643.

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

Mewes, H. et al. (2002). MIPS: a database for genomes and protein sequences. *Nucleic Acids Research* 30, 31–34.

Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D., and Alon, U. (2002). Network motifs: simple building blocks of complex networks. *Science* 298, 824–827.

Nabieva, E., Jim, K., Agarwal, A., Chazelle, B., and Singh, M. (2005). Whole-proteome prediction of protein function via graph-theoretic analysis of interaction maps. *Bioinformatics* 21, i302–i310.

Peña-Castillo, L. and Hughes, T. (2007). Why are there still over 1000 uncharacterized yeast genes? *Genetics* 176, 7–14.

Peri, S. et al. (2004). Human protein reference database as a discovery resource for proteomics. *Nucleic Acids Res* 32 Database issue, D497–501. 1362-4962 Journal Article.

Pržulj, N. (2006). Biological network comparison using graphlet degree distribution. *Bioinformatics* 23, e177–e183.

Pržulj, N., Corneil, D.G., and Jurisica, I. (2004). Modeling interactome: Scale-free or geometric? *Bioinformatics* 20(18), 3508–3515.

Pržulj, N., Wigle, D., and Jurisica, I. (2004). Functional topology in a network of protein interactions. *Bioinformatics* 20(3), 340–348.

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

Rual, J.F. et al. (2005). Towards a proteome-scale map of the human protein-protein interaction network. *Nature* 437, 1173–78.

Samanta, M. and Liang, S. (2003). Predicting protein functions from redundancies in large-scale protein interaction networks. *PNAS* 100, 12579–12583.

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

Stark, C., Breitkreutz, B., Reguly, T., Boucher, L., Breitkreutz, A., and Tyers, M. (2006). BioGRID: A general repository for interaction datasets. *Nucleic Acids Research* 34, D535–D539.

Sun, S. et al. (2006). Faster and more accurate global protein function assignment from protein interaction networks using the MFGO algorithm. *FEBS Letters* 580, 1891–1896.

Vazquez, A., Flammini, A., Maritan, A., and Vespignani, A. (2003). Global protein function prediction from protein-protein interaction networks. *Nature Biotechnology* 21, 697–700.

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

Xenarios, I., Salwinski, L., Duan, J., Higney, P., Kim, S., and Eisenberg, D. (2002). DIP, the database of interacting proteins: a research tool for studying cellular networks of protein interactions. *Nucleic Acids Research* 30, 303–305.