Identification of Protein Complexes by Comparative Analysis of Yeast and Bacterial Protein Interaction Data

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Abstract

Mounting evidence shows that many protein complexes are conserved in evolution. Here we use conservation to find complexes that are common to yeast *S. Cerevisiae* and bacteria *H. pylori*. Our analysis combines protein interaction data, that are available for each of the two species, and orthology information based on protein sequence comparison. We develop a detailed probabilistic model for protein complexes in a single species, and a model for the conservation of complexes between two species. Using these models, one can recast the question of finding conserved complexes as a problem of searching for heavy subgraphs in an edge- and node-weighted graph, whose nodes are orthologous protein pairs.

We tested this approach on the data currently available for yeast and bacteria and detected 11 significantly conserved complexes. Several of these complexes match very well with prior experimental knowledge on complexes in yeast only, and serve for validation of our methodology. The complexes suggest new functions for a variety of uncharacterized proteins. By identifying a conserved complex whose yeast proteins function predominantly in the nuclear pore complex, we propose that the corresponding bacterial proteins function as a coherent cellular membrane transport system. We also compare our results to two alternative methods for detecting complexes, and demonstrate that our methodology obtains a much higher specificity.

1 Introduction

With the sequences of dozens of genomes at hand, and the accumulating information on the transcriptomes and proteomes of different organisms, a new research paradigm is emerging in molecular biology. At the core of this paradigm is the comparative analysis of biological properties of two or more species, using the wealth of organisms to enhance weak relations and to draw conclusions on one species, based on available information on other species. Comparative analysis, in the form of pairwise alignment, is frequently used in predicting protein function and structure. Recently, comparative approaches have been proven useful in diverse domains, including gene finding [5], motif finding [12], cis-regulation [19] and metabolic pathways [15, 22].

Protein interactions are crucial to cellular function, both in assembling protein machinery and complexes and in signaling cascades, where protein interactions enable one protein to modify another to transmit biological information. Recent technological advances enable us for the first time

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to characterize networks of protein interactions. Among the most direct and systematic methods for measuring protein interactions are co-immunoprecipitation [13] and the two-hybrid system [9], that have defined large protein-protein interaction networks for organisms including *S. cerevisiae* [8], *H. pylori* [17], and *C. elegans* [6].

The growing information on protein networks for different organisms naturally lends itself to comparative analysis, which tries to determine the extent to which protein networks are conserved among species and make use of the discovered conservation to predict novel networks or parts of networks. Mounting evidence suggests that conserved protein interaction pathways indeed exist and may be ubiquitous: For example, proteins in the same pathway are typically present or absent in a genome as a group [16], and several hundred protein-protein interactions in yeast have also been identified for the corresponding protein orthologs in worms [14].

Previously, we have studied the conservation of pathways between the budding yeast *S. cerevisiae* and the bacterial pathogen *H. pylori* by performing a whole-network-based comparison between the protein-protein interaction networks of the two species [11]. Our analysis suggested many similarities between the two networks. Some of these similarities correspond to well-known biological pathways, while others represent new biological discoveries.

Here we study another aspect of the similarity between the yeast and bacterial protein networks, namely, the conservation of complexes between the two species. In both the present study and the earlier one the goal is to find two sets of proteins, one in yeast and one in bacteria, such that many of the proteins in each set have orthologous counterparts in the other, there is a high level of interaction among the proteins in each set, and the patterns of interaction in the two sets are similar. But while the methods of the earlier study were aimed at finding chain-like patterns of interaction of the type that might occur in signal transduction pathways, the present study focuses on protein complexes and looks for dense, clique-like interaction patterns that are conserved in the two species.

At the heart of our analysis is a novel probabilistic model for protein interaction network in a single species, and a two-species model, which combines, in a unified manner, the interaction model of each species as well as information on the similarity of protein pairs between the two species. This model is used to construct an *orthology graph*, whose nodes correspond to pairs of putative orthologs, and whose edges correspond to protein interactions. The edges of the graph are assigned weights with probabilistic meaning, so that high weight subgraphs correspond to conserved protein complexes. We propose a practical method to search the orthology graph for complexes of densely interacting proteins, which is based on forming high weight seeds and extending them using local search.

We applied our algorithm to data on protein interactions and protein sequences for yeast and bacteria. The algorithm identified 11 significantly conserved complexes, with sizes ranging between 6 and 20 protein pairs. Several of these complexes match very well with prior experimental knowledge on complexes in yeast only, and serve for validation of our methodology. The identified complexes suggest new functions for a variety of uncharacterized proteins. In particular, by identifying a conserved complex whose yeast proteins function predominantly in the nuclear pore complex, we propose that the corresponding bacterial proteins function as a coherent cellular membrane transport system. We also compared our results to two alternative methods for detecting complexes, and demonstrated that our methodology obtains a much higher specificity. In addition, we showed the utility of using our probabilistic model for analyzing interaction data from a single species.

The paper is organized as follows: In Section 2 we present our probabilistic model for comparative protein interaction data. In Section 3 we describe our algorithm for identifying high-scoring, conserved protein complexes. The application to real data and comparison to extant approaches

are presented in Section 4.

2 A Probabilistic Model for Protein Complexes

In this section we present a probabilistic model for protein interaction data within a single species, and then extend it to two species. Given a dataset of protein interactions of some organism, we translate it into an *interaction graph* G, whose vertices are the organism's interacting proteins, and whose edges represent pairwise interactions between distinct proteins. Using this formulation, a protein complex corresponds to a subgraph of G that is typically dense. Hence, surprisingly dense subgraphs in G may be suggested as putative protein complexes.

In the case of perfect data, each edge in the interaction graph represents a known interaction, each non-edge represents a known non-interacting pair, and we are seeking a surprisingly dense subgraph of G. To this end we formulate a log likelihood ratio model that is additive over the edges and non-edges of G, such that highly scoring subgraphs would correspond to likely protein complexes. Such a model requires specifying a null model and a protein-complex model for vertex pairs. Similarly to the probabilistic approach taken in [20], we define the two models as follows: The *protein-complex model*, M_c, assumes that every two proteins in a complex interact with some high probability β. In terms of the graph, the assumption is that two vertices that belong to the same complex are connected by an edge with probability β. While our model assumes a clique structure of a protein complex, other reasonable models could be formulated, such as a "hub" model, in which all vertices connect to a center vertex of high degree.

In contrast, the *null model*, M_n, assumes that each edge is present with the probability that one would expect if the edges of G were randomly distributed but respected the degrees of the vertices. More precisely, we let F^G be the family of all graphs having the same vertex set as G and the same degree sequence, and we define the probability of observing the edge (u, v) to be the fraction of graphs in F^G that include this edge. Note that in this way, edges incident on vertices with higher degrees have higher probability.

A complicating factor in constructing the interaction graph is that we do not know the real protein interactions, but rather have partial, noisy observations of these interactions. Formally, let us denote by T_{uv} the event that two proteins u, v interact, and by F_{uv} the event that they do not interact. Denote by O_{uv} the (possibly empty) set of available observations on the proteins u and v, that is, the set of experiments in which an interaction between u and v was, or was not, observed. Using prior biological information one can estimate for each protein pair the probability $Pr(O_{uv}|T_{uv})$ of the observations on this pair given that it interacts, and the probability $Pr(O_{uv}|F_{uv})$ of our observations given that this pair does not interact. Also, one can estimate the prior probability $Pr(T_{uv})$ that two random proteins interact.

Given a subset U of the vertices, we wish to compute the likelihood of U under a protein-complex model and under a null model. Denote by O_U the collection of all observations on vertex pairs in U. Then

$$
\begin{aligned}
Pr(O_U|M_c) &= \prod_{(u,v)\in U\times U} Pr(O_{uv}|M_c) && (1)\\
&= \prod_{(u,v)\in U\times U} [Pr(O_{uv}|T_{uv}, M_c)Pr(T_{uv}|M_c) + Pr(O_{uv}|F_{uv}, M_c)Pr(F_{uv}|M_c)] && (2)\\
&= \prod_{(u,v)\in U\times U} [\beta Pr(O_{uv}|T_{uv}) + (1-\beta)Pr(O_{uv}|F_{uv})] && (3)
\end{aligned}
$$

Equation 1 follows from the assumption that all pairwise interactions are independent. Equation 2

is obtained using the law of complete probability. Equation 3 follows by noting that given the hidden event of whether u and v interact, O_{uv} is independent of any model.

It remains to compute $Pr(O_U|M_n)$. Since our previous null model depended on having the degree sequence of the interaction graph, we cannot use it as is. To overcome this difficulty we approximate the degree sequence of the hidden interaction graph: Let $d_1, \ldots, d_n$ denote the expected degrees of the vertices in G, rounded to the closest integer. In order to compute the expected degrees we apply Bayes' rule to derive the expectation of every vertex pair:

$$Pr(T_{uv}|O_{uv}) = \frac{Pr(O_{uv}|T_{uv})Pr(T_{uv})}{Pr(O_{uv}|T_{uv})Pr(T_{uv}) + Pr(O_{uv}|F_{uv})(1 - Pr(T_{uv}))}$$

Our refined null model assumes that G is drawn uniformly at random from the collection of all graphs, whose degree sequence is $d_1, \ldots, d_n$. This induces a probability p_{uv} for every vertex pair (u, v). We can now calculate the probability of O_U according to the null model:

$$Pr(O_U|M_n) = \prod_{(u,v)\in U\times U} [p_{uv}Pr(O_{uv}|T_{uv}) + (1 - p_{uv})Pr(O_{uv}|F_{uv})]$$

Finally, the log likelihood ratio that we assign to a subset of vertices U is

$$\begin{aligned} L(U) &= \log \frac{Pr(O_U|M_c)}{Pr(O_U|M_n)} && (4)\\ &= \sum_{(u,v)\in U\times U} \log \frac{\beta Pr(O_{uv}|T_{uv}) + (1-\beta)Pr(O_{uv}|F_{uv})}{p_{uv}Pr(O_{uv}|T_{uv}) + (1-p_{uv})Pr(O_{uv}|F_{uv})} && (5)\\ &= \sum_{(u,v)\in U\times U} \log \frac{\beta Pr(T_{uv}|O_{uv})(1-Pr(T_{uv})) + (1-\beta)(1-Pr(T_{uv}|O_{uv}))Pr(T_{uv})}{p_{uv}Pr(T_{uv}|O_{uv})(1-Pr(T_{uv})) + (1-p_{uv})(1-Pr(T_{uv}|O_{uv}))Pr(T_{uv})} && (6) \end{aligned}$$

where Equation 6 follows by applying Bayes' rule and cancelling common terms in the numerator and denominator.

2.1 Two-Species Conservation Model

Consider now the case of data on two species 1 and 2, denoted throughout by an appropriate superscript. Here we wish to score a conserved complex that is defined by two subsets of proteins, one from each species, and a many to many correspondence associating proteins in one species with their orthologous proteins in the other species. Consider two subsets $U^1 = \{u^1_1, \ldots, u^1_{k_1}\}$ and $V^2 = \{v^2_1, \ldots, v^2_{k_2}\}$ and some mapping $\theta : U^1 \to V^2$ between them. Assuming that the interaction graphs of the two species are independent of each other, the log likelihood ratio score for these two sets is simply:

$$L(U^1, V^2) = \log \frac{Pr(O_{U^1}|M^1_c)}{Pr(O_{U^1}|M^1_n)} + \log \frac{Pr(O_{V^2}|M^2_c)}{Pr(O_{V^2}|M^2_n)}$$

However, this score does not take into account the degree of sequence conservation among the pairs of proteins associated by θ. In order to include such information we have to define a conserved complex model and a null model for pairs of proteins from two species. Our conserved complex model assumes that pairs of proteins associated by θ are orthologous. The null model assumes that such pairs consist of two independently chosen proteins. Let E_{uv} denote the BLAST E-value assigned to the similarity between proteins u and v, and let $h_{uv}, \bar{h}_{uv}$ denote the events that u and

v are orthologous, or non-orthologous, respectively. The likelihood ratio corresponding to a pair of proteins (u, v) is therefore

$$\frac{Pr(E_{uv}|M_c)}{Pr(E_{uv}|M_n)} = \frac{Pr(E_{uv}|h_{uv})}{Pr(E_{uv}|h_{uv})Pr(h_{uv}) + Pr(E_{uv}|\bar{h}_{uv})Pr(\bar{h}_{uv})}$$

Using Bayes' rule we get that this ratio is simply $\frac{Pr(h_{uv}|E_{uv})}{Pr(h)}$, where $Pr(h)$ is the prior probability that two proteins are orthologous.

Thus, the complete score of U^1 and V^2 under the mapping θ is:

$$S_\theta(U^1, V^2) = L(U^1, V^2) + \sum_{i=1}^{k_1} \sum_{v_j^2 \in \theta(u_i^1)} \log \frac{Pr(h_{u_i^1 v_j^2}|E_{u_i^1 v_j^2})}{Pr(h)}$$

3 Searching for Conserved Complexes

Using the above model for comparative interaction data, the problem of identifying conserved protein complexes reduces to the problem of identifying a subset of proteins in each species, and a correspondence between them, such that the score of these subsets exceeds a threshold. This problem is NP-hard even when considering a single species where all edge weights are 1 or -1 and all vertex weights are 0 [18]. Thus, in the following, we propose heuristic strategies for the search problem.

To allow efficient search for conserved protein complexes, we define a complete weighted *orthology graph* (extending [11]). We focus on yeast and bacteria. Denote by superscripts p and y the model parameters corresponding to bacteria and yeast, respectively. For two yeast proteins y_1 and y_2 define

$$w^y_{(y_1,y_2)} = \log \frac{\beta^y Pr(O_{y_1y_2}|T^y_{y_1y_2}) + (1-\beta^y)Pr(O_{y_1y_2}|F^y_{y_1y_2})}{p^y_{y_1y_2} Pr(O_{y_1y_2}|T^y_{y_1y_2}) + (1-p^y_{y_1y_2})Pr(O_{y_1y_2}|F^y_{y_1y_2})}$$

Similarly, for two bacterial proteins p_1 and p_2 define

$$w^p_{(p_1,p_2)} = \log \frac{\beta^p Pr(O_{p_1p_2}|T^p_{p_1p_2}) + (1-\beta^p)Pr(O_{p_1p_2}|F^p_{p_1p_2})}{p^p_{p_1p_2} Pr(O_{p_1p_2}|T^p_{p_1p_2}) + (1-p^p_{p_1p_2})Pr(O_{p_1p_2}|F^P_{p_1p_2})}$$

Every pair (y_1, p_1) of yeast and bacterial proteins is assigned a *node*, whose weight reflects the similarity of the proteins, that is,

$$w_{(y_1,p_1)} = \log \frac{Pr(h|E_{y_1p_1})}{Pr(h)} \ .$$

Every two distinct (but possibly overlapping) nodes $(y_1, p_1), (y_2, p_2)$, are connected by an edge, which is associated with a pair of weights $(w^y_{(y_1,y_2)}, w^p_{(p_1,p_2)})$. If $y_1 = y_2$ ($p_1 = p_2$) we set the first (second) coordinate to 0. The edge is called *strong* if the sum of its associated weights is positive.

By construction, an induced subgraph of the orthology graph corresponds to two subsets of proteins, one from each species, and a many to many correspondence between them. We define the *z-score* of an induced subgraph with vertex sets U^1 and V^2 and a mapping θ between them, as the log likelihood ratio score $S_\theta(U^1, V^2)$ for the subgraph, normalized by subtracting its mean and dividing by its standard deviation. In computing the z-score we assume that node- and edge-weights are independent, so the mean and variance of $S_\theta(U^1, V^2)$ are obtained by summing the means and variances of the corresponding nodes and edges. High-scoring induced subgraphs in the orthology graph correspond to putative conserved protein complexes.

In order to reduce the complexity of the graph and focus on biologically plausible conserved complexes, we filter nodes from the graph as follows: We start with an initial set of yeast-bacterial protein pairs, whose BLAST E-value is smaller than 10^{-2}. We filter from this set pairs for which at least one of the proteins has no interactions with other proteins (in our data). Let S be the resulting set of pairs. Pairs of proteins that do not belong to S are considered to have low likelihood to take part in a conserved complex [11]. Hence, we consider them only if they satisfy the following condition: For every node $(p, y) \notin S$ we check whether there exist two nodes $(p_1, y_1), (p_2, y_2) \in S$ such that p interacts with p_1 and p_2 and y interacts with y_1 and y_2. If no such nodes exist, we remove (p, y) from the graph. Otherwise, we retain it. The added nodes serve as 'bridges' in the orthology graph between protein pairs, whose members in each species are not known to directly interact.

Next, we perform a bottom-up search for heavy subgraphs in the orthology graph. We start from high weight seeds, refine them by exhaustive enumeration, and then expand them using local search. A similar approach based on local search was shown to work well in analyzing high-throughput genomic data [20]. In the first phase of the search we compute a seed around each node v, which consists of v and all its neighbors u such that (u, v) is a strong edge. If the size of this set is above a threshold (e.g., 10) we iteratively remove from it the node whose contribution to the subgraph score is minimum, till we reach the desired size. Next, for each seed S we enumerate all subsets of S of size at least 3 that contain v. Each such subset is a refined seed on which we apply a local search heuristic. In the local search we iteratively add a node, whose contribution to the current seed is maximum, or remove a node whose contribution to the current seed is minimum, as long as this operation increases the overall score of the seed. In the process we preserve the original seed and do not delete nodes from it. For each node in the orthology graph we record up to k (e.g., 5) heaviest subgraphs that were discovered around that node.

The resulting subgraphs may overlap considerably, so we use a greedy algorithm to filter subgraphs whose percentage of intersection is above a threshold. The algorithm iteratively finds the highest weight subgraph, adds it to the final output list, and removes all other intersecting subgraphs (see Section 4 for precise details on the filtering criterion).

In order to evaluate the statistical significance of identified complexes we compute two kinds of p-values. The first is based on the z-scores that we compute, and relies on a normal approximation to the score of a subgraph, assuming that its nodes and edges contribute independent terms to the score. The latter probability is Bonferroni corrected for multiple testing, according to the size of the subgraph. The second is based on empirical runs on randomized data. The randomized data is produced by random shuffling of the original interaction graphs of the two species, preserving their degree sequences. For each randomized dataset we use our heuristic search to find the highest-scoring conserved complex of a given size. We then estimate the p-value of a suggested complex of the same size, as the fraction of random runs in which the output complex had larger score.

4 Experimental Results

4.1 Building the Orthology Graph and Parameter Estimation

Our goal was to find conserved complexes between *S. cerevisiae* and *H. pylori*. We first constructed a protein-protein interaction network for each species. The yeast network contained 14,848 pairwise interactions among 4,716 proteins, and is based on several systematic studies using protein co-immunoprecipitation and the yeast two-hybrid system. The bacterial network contained 1,403 pairwise interactions among 732 proteins, which come from a single two-hybrid study [17]. All interactions were extracted from DIP [25] (August 2003 version).

Protein sequences for both species were obtained from PIR [24]. Alignments and associated E-values were computed using BLAST 2.0 [3] with parameters $b = 0$; $e = 1E6$; $f =$"C;S"; $v = 6E5$. Altogether, 1909 protein pairs had E-value below 0.01, out of which 822 pairs contained proteins with some measured interaction. Adding 1242 additional pairs with weak homology (as described in Section 3, and removing nodes with no incident strong edge, resulted in a final orthology graph G with 866 nodes and 12,420 edges. In total, 248 distinct bacterial proteins and 527 yeast proteins participated in these nodes.

A good estimation of the probabilistic parameters in the model is a precondition to obtaining meaningful results. We computed the parameters based on an estimate of 6,334 proteins and 20,000 true protein-protein interactions in yeast [4]. We used the maximum likelihood method of Deng et al. [7] for estimating the reliability of observed interactions. The method provides reliability estimates that depend on the experimental method used to detect the interaction, and on the number of times each interaction was observed. We did not use negative information on interactions that were tested but were not observed, as such data was not readily available. The prior probability that a yeast protein and a bacterial protein are orthologous was computed as the frequency of protein pairs from both species that are in the same COG (cluster of orthologous genes) [21] (see also [11]). The conditional probability that a pair of proteins are orthologous, given their BLAST E-value, was computed as in [11]. For each species, the probabilities of observing each particular edge in a random graph with the same degree sequence, was computed by Monte-Carlo simulations as follows. Starting from the original interaction graph, we performed a long series of random edge crosses, each time picking at random two edges $(a, b), (c, d)$, and replacing them with $(a, c), (b, d)$ (disallowing self-loops), provided that the latter two edges were not present in the current graph. The percentage of simulations in which an edge was observed was the estimate of its probability. The concrete values of the parameters are listed in the appendix.

4.2 Identifying Conserved Protein Complexes

We applied our algorithm to the yeast-bacteria orthology graph in search of conserved complexes. Altogether, the algorithm identified 11 non-redundant complexes, whose p-values were smaller than 0.05, after correction for multiple testing. These complexes were also found to be significant, when scored against empirical runs on randomized data ($p < 0.05$). The complexes are listed in Table 4.2. Complex sizes varied between 6 to 20 protein pairs (20 was the maximum allowed size). Redundant complexes were filtered by disallowing large overlap between two complexes. Precisely, if 60% of the nodes or 60% of the distinct proteins in each species were common to two complexes, the one with the poorer p-value was removed.

To validate the results, we first used information about known protein complexes in yeast. We extracted assignments of yeast genes to complexes from the MIPS database [1] (August 2003 version). 285 nodes in the orthology graph had such assignments. We used complex categories at level 3 of the MIPS complex hierarchy. In total, 18 categories had at least three genes from the orthology graph, and six categories had at least five. For each of our complexes we computed the largest number of proteins from a single category, as a fraction of all its categorized members. This fraction is called the *purity* of the complex. High purity indicates a conserved complex that corresponds to a known complex in yeast, and serves as a validation for the result. Low purity may either indicate an incorrect complex or a previously unidentified correct one. Note that most complexes also contain proteins that are not known to belong to any complex in yeast, and so our results suggest additional members in known complexes.

For bacteria, since experimental information on complexes is unavailable, we used functional annotations instead. We extracted 864 functional annotations of bacterial genes from the TIGR

database [2]. We used a categorization of *H. pylori* genes into 13 broad functional classes, spanning 757 nodes in our orthology graph. Purity was computed in the same manner.

Our conserved protein complexes suggest new functions for a variety of uncharacterized proteins. For instance, complex 17 (Figure 1(a)) defines a set of conserved interactions for the cell's protein degradation machinery. Bacterial proteins HP0849 and HP0879 are largely uncharacterized, but their appearance among yeast and bacterial proteins involved in proteolysis suggests that they also play an important role in this process. Furthermore, it appears that the yeast proteins Hsm3 and Rfa1 (with known functional roles in DNA-damage repair) may also be associated with the yeast proteasome. Complexes 19 and 31 (Figure 1(b,d)) suggest that their component proteins, some of which are uncharacterized, are involved in protein synthesis.

As another example of protein functional prediction, Figure 1(b) shows a conserved complex which contains yeast proteins that function in the nuclear pore (NUP) complex. The NUP complex is integral to the eukaryotic nuclear membrane and serves to selectively recognize and shuttle molecular cargos (e.g., proteins) between the nucleus and cytoplasm. Unlike the yeast proteins, the corresponding bacterial proteins are less well characterized, although three have been associated with the cell envelope due to their predicted transmembrane domains. Our results therefore indicate that the bacterial proteins may function as a coherent cellular membrane transport system in bacteria, similar to the nuclear pore in eukaryotes. Although further experimentation will be necessary to explore this hypothesis, it is possible that these proteins comprise the ancestral prokaryotic machinery from which the NUP transport system evolved.

ID	Score	Size	Yeast enrichment		Bacterial enrichment	
			Purity	Complex Category	Purity	Functional Category
1	16.16	12 (12,10)	0.17 (1/6)	Translation (1)	0.56 (5/9)	DNA-metabolism (7)
8	3.31	6 (6,6)	1.00 (4/4)	Respiration (4)	0.33 (2/6)	Energy-metabolism (71)
17	141.31	12 (6,12)	0.90 (9/10)	Proteasome (9)	0.50 (2/4)	Protein-synthesis (30)
18	37.31	13 (9,13)	0.45 (5/11)	Proteasome (9)	0.25 (2/8)	DNA-metabolism (7)
19	19.09	6 (6,6)	1.00 (6/6)	Translation (10)	0.80 (4/5)	Protein-synthesis (30)
25	40.16	10 (8,10)	0.67 (4/6)	Replication (4)	0.20 (1/5)	Energy-metabolism (71)
28	9.39	9 (9,9)	0.60 (3/5)	Translation (10)	0.50 (4/8)	Protein-synthesis (30)
30	383.52	20 (12,20)	0.55 (6/11)	NUP (6)	0.43 (3/7)	Cell-envelope (27)
31	7.21	6 (6,6)	0	-	1.00 (4/4)	Protein-synthesis (30)
32	3.05	7 (6,7)	0.67 (2/3)	Transcription (3)	0.25 (1/4)	Transcription (13)
33	15.68	13 (12,12)	0.40 (2/5)	RNA-processing (2)	0.33 (3/9)	DNA-metabolism (7)

Table 1: Conserved protein complexes identified between yeast and bacteria. For each complex the table lists its score (-log p-value, adjusted for multiple testing); its size (with the numbers of distinct bacterial and yeast proteins in parentheses); purity, as measured using MIPS level 3 categorization of complexes in yeast (with the number of proteins from the most abundant category, and the total number of categorized proteins in the complex, in parentheses); the most abundant category (and its size in parentheses); functional purity, as measured using functional annotation in bacteria; and the most abundant class (with its size in parentheses). A zero enrichment for a complex indicates that there is at most one annotated member of the complex. Abbreviations: NUP (nuclear pore complex).

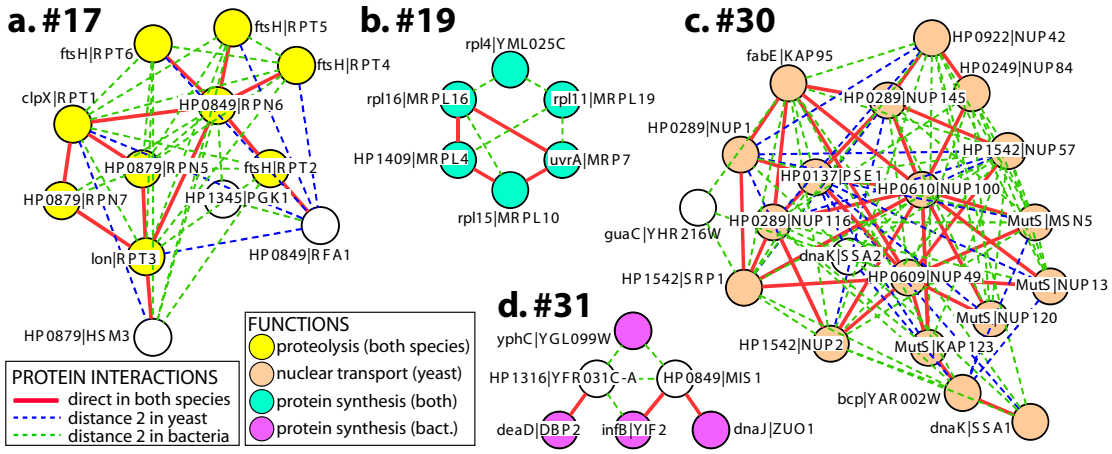

Figure 1: Conserved protein complexes for proteolysis (panel a), protein synthesis (panels b and d), and nuclear transport (panel c). Conserved complexes are connected subgraphs within the bacteria/yeast orthology graph, whose nodes represent orthologous protein pairs and edges represent conserved protein interactions of three types: Direct interactions in both species (red edges); direct in bacteria but distance 2 in the yeast interaction graph (blue edges); and distance 2 in the bacterial interaction graph but direct in yeast (green edges). In the algorithm, both nodes and edges are assigned weights according to the probabilistic model. The number of each complex indicates the corresponding complex ID listed in Table 4.2.

4.3 Comparisons to Extant Approaches

In order to assess the advantage of our approach, we searched for complexes in the data using two other methods: First, we formed a variant of our algorithm which uses only the protein-protein interactions in yeast, and searched for yeast complexes. This test was aimed at seeing what is gained (and lost) by using the constraint of cross-species conservation. Second, we tested our previous probabilistic model for protein interactions [11]. That latter model is much less involved than the current one: The weight of each vertex in the orthology graph is set to the logarithm of the probability that the member proteins are orthologous. The weight of an edge is set to the logarithm of the probability that it represents a true interaction.

We used three measures to compute the quality of the results. All three quantify the similarity between a given solution and a reference, putatively true, solution. In our case, we used the known complex categories in yeast as the reference solution, since no knowledge on *conserved* complexes is available. The *Jaccard* measure, which is often used in clustering (cf. [10]), uses the notion of mates. Two proteins are called *mates* in a solution if they appear together in at least one complex in that solution. Given two solutions, let n_{11} be the number of pairs that are mates in both, and let n_{10} (n_{10}) be the number of pairs that are mates in the first (second) only. The Jaccard score is $n_{11}/(n_{11}+n_{10}+n_{01})$. Hence, it measures the correspondence between protein pairs that belong to a common complex according to one or both solutions. Two identical solutions would get a score of 1, and the higher the score the better the correspondence. The *sensitivity* measure quantifies the extent to which a solution captures complexes from the different yeast categories. It is formally defined as the number of categories for which there was a complex with at least half

the annotated elements in the category, divided by the number of categories with at least three annotated proteins. The *specificity* measure quantifies the accuracy of the solution. Formally, it is the fraction of predicted complexes whose purity exceeded 0.5.

A comparison of the performance the three approaches is presented in Table 4.3. The Jaccard score is significantly better in our current approach than in [11]. The sensitivity is lower, as we capture fewer categories, but the specificity is much higher, so our predicted complexes are much more accurate. Interestingly, when applying our algorithm using only data on yeast we get even higher sensitivity, although again at the cost of specificity. The Jaccard score of this run is comparable to that of the comparative algorithm. This shows that our new probabilistic model can be effectively used, even for detecting complexes using interaction data from a single species. Note that we evaluated the results using data on yeast complexes only, not all of which are expected to be conserved. Still, the use of the bacterial data significantly improved the specificity of the results.

Algorithm	Jaccard	Sensitivity	Specificity
This study	0.32	0.33	0.7
Kelley et al. [11]	0.22	0.44	0.4
Yeast only	0.33	0.67	0.48

Table 2: Performance comparison of three algorithms for complex detection.

Conclusions

We have presented a novel probabilistic model for the detection of conserved complexes among two species, and an algorithm to search for significant complexes. We applied our approach to study the conservation between yeast and bacterial protein networks. We identified highly specific complexes that were validated using known complexes in yeast and functional annotation in bacteria. Although the present work has already revealed several conserved biological structures that may have functional significance, many refinements and extensions to our method should be explored. Our model can be readily extended to allow interactions between two domains of the same protein (manifested as self-loops in the interaction graph). Models in which the primitive elements are domains within proteins, rather than entire proteins, may be of value. We have used a dense subgraph model that tends to find clique-like patterns of interaction; variations of the model oriented towards other kinds of interaction patterns are also of interest. Protocols such as the two-hybrid system detect directed interactions between proteins, suggesting the use of a directed or mixed interaction graph instead of the current undirected model. In order to find complexes conserved in k species, where $k > 2$, our models should be extended to k-species orthology graphs, in which each node specifies a protein from each of k species; the scoring of such nodes is an open question. Negative data, indicating the absence of protein-protein interactions, should be used to supplement the positive data presently used. Co-expression of genes, as measured in microarray experiments, can also provide indirect evidence for the interaction between the corresponding proteins. Finally, the current protein-protein interaction data is sparse and unreliable; as the abundance and quality of the data improve, the predictive power of our methods and their future refinements will be greatly enhanced.

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A Model Parameters

In the following we detail the parameters that we used as input to our probabilistic model:

- The probability of observing an interaction in a complex model was set both in yeast and bacteria to $\beta = 0.95$.
- The prior probability for a true orthology among yeast and bacterial proteins, whose evaluation is described in Section 4.1, was set to $Pr(h) = 0.001611$.
- The prior probability of observing some interaction for a given pair of vertices was set according to the number of interactions and proteins in our data: $Pr(O^y_{y_1,y_2} \neq \emptyset) = 0.00135$ and $Pr(O^p_{p_1,p_2} \neq \emptyset) = 0.00524$.
- The prior probability for a true interaction, whose estimation is described in Section 4.1, was set to 0.001, for both species. The probability of observing a true interaction was estimated by the ratio of expected number of true interactions that were observed and the number of true interactions.
- The reliability of the interactions in *H. pylori* was estimated at 0.53, which was the reliability assigned by Deng et al. [7] to the yeast two-hybrid experiment of Uetz et al. [23], and was supported by the estimations given in [17].