Comparison of Protein-Protein Interaction Confidence Assignment Schemes

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Abstract. Recent technological advances have enabled high-throughput measurements of protein-protein interactions in the cell, producing protein interaction networks for various species at an ever increasing pace. However, common technologies like yeast two-hybrid can experience high rates of false positive detection. To combat these errors, many methods have been developed which associate confidence scores with each interaction. Here we perform the first comparative analysis and performance assessment among these different methods using the fact that interacting proteins have similar biological attributes such as function, expression, and evolutionary conservation. We also introduce a new measure, the signal to noise ratio of protein complexes embedded in each network, to assess the quality of the different methods. We observe that utilizing any probability scheme is always more beneficial than assuming all observed interactions to be real. Also, schemes that assign probabilities to individual interactions generally perform better than those assessing the reliability of a set of interactions obtained from an experiment or a database.

1 Introduction

Systematic elucidation of protein-protein interaction networks will be essential for understanding how different behaviors and protein functions are integrated within the cell. Recently, the advent of high-throughput experimental techniques like yeast two-hybrid [1] assays and mass spectrometry [2] has lead to the discovery of large-scale protein interaction networks in different species, including *S. cerevisiae* [2-5], *D. melanogaster* [6], *C. elegans* [7] and *H. sapiens* [8, 9]. Unfortunately, these large-scale data sets have so far been generally incomplete and associated with a significant number of false-positive interactions [10]. However, recent years have also seen an increase in the accumulation of other sources of biological data such as whole genome sequence, mRNA expression, protein expression and functional annotation. This is particularly advantageous as some of these data sets can be utilized to reinforce true (physical) protein interactions while downgrading others. For instance, true protein interactions have been shown to have high mRNA expression correlation for the proteins involved [11].

As a result, many bioinformatics approaches have been developed to unearth true protein interactions which can be mainly divided into two categories: (1) methods that assign reliability measurements to previously observed interactions; and (2) methods

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that predict interactions *ab initio*. For category (1), Deane *et al*. [12] introduced one of the first methods to tackle the problem of assigning reliabilities to interactions using similarity in mRNA expression profiles. Subsequently, Bader *et al.* [13] and Deng *et al.* [14] used additional features of interacting proteins, including functional similarity and high network clustering [15], to assign confidence scores to protein interactions. For category (2), Marcotte *et al.* [16], von Mering *et al.* [17] and Jansen *et al.* [18] were among the first to predict new protein interactions by incorporating a combination of different features like high mRNA expression correlation, functional similarity, co-essentiality, and co-evolution. These schemes calculate a log-likelihood score for each interaction.

Here, we perform the first benchmarking analysis to compare the different interaction probability assignment schemes versus one another. We limit ourselves to methods that assign probabilities to interactions as opposed to those that compute a log-likelihood ratio. We also assess each of the methods against a "null hypothesis", a uniform scheme which considers the same probability for all interactions. To compare the quantitative accuracy of the methods, we examine the correlations between the probability estimations and different biological attributes such as function and expression. As a further comparison criterion, we introduce and apply the signal processing concept of 'Signal to Noise ratio' (SNR) to evaluate the significance of protein complexes identified in the network based on the different schemes The discovery of these complexes depends on the connectivity of the interaction network which is determined by the underlying interaction probability scheme [19]. Finally, we compare the different weighting schemes based on previous observations regarding the preference of interacting proteins to have similar conservation characteristics [20, 21].

2 Interaction Confidence Assignment Schemes

Although large-scale protein interaction networks are being generated for a number of species, *S. cerevisiae* (yeast) is perhaps the best studied among them and is associated with the largest variety and number of large-scale data. Hence, most of the interaction probability schemes have been developed specifically for the yeast protein interaction network. The yeast network was also the focus of our analysis in which we considered interaction probability scores by Bader *et al*. [13], Deane *et al.* [12], Deng *et al.* [14], Sharan *et al.* [19] and Qi *et al.* [22]. Bader *et al.,* Sharan *et al.* and Qi *et al.* assigned specific probabilities to every interaction, while Deane *et al*. and Deng *et al.* grouped the interactions into high/medium/low confidence groups. All of the above schemes estimated the predicted reliabilities of each interaction based on a gold standard set of positive and negative interaction data. Specifically, each weighting scheme defined gold standard sets based on various biological observations.

2.1 Bader et al. (BL / BH)

As a gold standard positive training data set, Bader *et al.* [13] used interactions determined by co-immunoprecipitation (co-IP), in which the proteins were also one or two links apart in the yeast two-hybrid (Y2H) network. The negative training data set

was selected from interactions reported either by co-IP or Y2H, but whose distance (after excluding the interaction) was larger than the median distance in Y2H or co-IP respectively. Using these training data, they constructed a logistic regression model which computes the probability of each interaction based on explanatory variables including data source, number of interacting partners, and other topological features like network clustering. We refer to this scheme as Bader *et al.* (low) or BL in our analysis.

Initially, the authors used measures based on Gene Ontology (GO) [24] annotations, co-expression, and presence of genetic interactions as measures to validate their data. However, they also combined these measurements into the probability score to bolster their confidence of true interactions. We consider these new confidence scores in our analysis as Bader *et al.* (high) or BH.

2.2 Deane et al. (DE)

Deane *et al.* [12] estimated the reliability of protein-protein interactions using the expression profiles of the interacting partners. Protein interactions observed in small-scale experiments and also curated in the Database of Interacting Proteins (DIP) [25] were considered as the gold standard positive interactions. As a gold standard negative, they randomly picked protein pairs from the yeast proteome that were not reported in DIP. The authors used this information to compute the reliabilities of groups of interactions (obtained from an experiment or a database). Higher reliabilities were assigned to groups whose combined expression profile was closer to the gold standard positive than the gold standard negative interactions. Specifically, reliabilities were assigned to the whole DIP database, the set of all protein interactions generated in any high-throughput genome screen, and protein interactions generated by Ito *et al.* [4]

2.3 Deng et al. (DG)

Deng *et al*. [14] estimated the reliabilities of different interaction data sources in a manner similar to Deane *et al.* [12]. They separately considered experiments that report pair-wise interactions like Y2H and those that report complex membership like mass spectrometry. Curated pair-wise interactions from the literature and membership in protein complexes from MIPS [23] were used as the gold standard positive set in each case. Randomly chosen protein pairs formed the gold standard negative data set. Reliabilities for each data source were computed using a maximum likelihood scheme based on the expression profiles of each data set. The authors evaluated reliabilities for Y2H data sources like Uetz *et al.* [5] and Ito *et al.* [4], and protein complex data sources like Tandem Affinity Purification (TAP) [2] and high-throughput mass spectrometry (HMS) [3]. In addition to assigning reliabilities to each dataset, the authors also provided a conditional probability scheme to compute probabilities for each interaction. We use the probabilities generated by this method for our comparative analysis.

2.4 Sharan et al. (SH)

Recently, we have also implemented an interaction probability assignment scheme [19] similar to the one proposed by Bader *et al.* The scheme assigned probabilities to interactions using a logistic regression model based on mRNA expression, interaction

clustering and number of times an interaction was observed in independent experiments. Here we use a modification of this scheme, assigning probabilities to interactions based only on experimental evidence. Specifically, interactions with at least two literature references or those that had a distance 2 in both the co-IP and Y2H networks were defined as the gold standard positives. Conversely, proteins at a distance > 4 in the entire network (after removing the interaction in question) were defined as the gold standard negatives. Binary variables were used to denote whether the interaction was reported in a co-IP data set, Y2H data set, a small-scale experiment or a large–scale experiment. Interaction probabilities were then estimated using logistic regression on the predictor parameters as in Bader *et al.*

2.5 Qi et al. (QI)

In this study, the authors use the interactions which were observed in small-scale experiments and reported by either DIP or Bader *et al.* as their gold standard positive training data [22]. Randomly picked protein pairs were used as the gold standard negative training data. The method incorporates direct evidence such as the type of experiment used to generate the data (mass spectrometry, yeast two-hybrid, etc.) and indirect evidence like gene expression, existence of synthetic lethal interactions, and domain-domain interactions to construct a random forest (a collection of decision trees). The resulting forest is then used to calculate the probability that two proteins interact.

2.6 Average (AVG)

We computed a new weighting scheme based on the average probability assigned by all above schemes. This averaged weighting scheme was also used in our comparison.

2.7 Equal Probabilities (EQ)

Finally, we also considered the case in which all observed interactions were considered to be equally true. We refer to this case as Equal in the analysis.

A summary of all attributes used as input in the different probability schemes is provided in Table 1.

Table 1. Summary of input attributes for the different probability schemes. GO: Gene Ontology; DDI: Domain-domain Interactions; Nbrhd: Neighborhood; SL: Synthetic Lethal.

Prob. Scheme	Experiment Type	Protein-DNA binding	Gene / Protein Expression	Interaction Clustering	SL	GO	DDI	Gene Fusion / Co-occur / Nbrhd.
BL	*			*				
BH	*		*	*	*	*		
DE			*					
DG	*		*					
SH	*							
QI	*	*	*	*	*	*	*	*
EQ								

3 Assessment of Interaction Schemes

As the probability schemes were previously computed for different subsets of yeast PPIs, we first compiled a set of 11,883 interactions common to all schemes. Five measures that have been shown to be associated with true protein interactions were used to assess the accuracy of each interaction probability scheme. In some cases, one of the measures used to assess a schemes' performance was already used as an input in assigning the probabilities. Although this creates some amount of circularity, the measure remains useful for gauging the performance of the remaining probability schemes. For each of the six measures, we evaluated two ways to estimate the level of association: Spearman correlation, and mutual information. The Equal probability scheme results in a spearman correlation and mutual information values of 0, by default. Consequently, we also evaluate the weighted average for each probability scheme. The weighted average is given by $WA = \frac{\sum_{i=1}^{N} p_i * m_i}{\sum_{i=1}^{N} p_i}$, where p_i is the probability of a given interaction and m_i is the value of one of the five measures for the interaction.

3.1 Global Properties of Interaction Probability Schemes

As a first measure, we compared global statistics such as the average and median probability assigned by each scheme and the number of interactions p 0.5 (Table 2). We also computed Spearman correlations among the different probability schemes to measure their level of inter-dependency (Table 3). The maximum correlation was seen between BL and BH, as might be expected as both schemes were reported in the same study and BH was derived from BL. In addition, we also evaluated the spearman correlation between the measures used to assess accuracy of the probability schemes (see Appendix).

Table 2. Comparison of Global properties of different probability assignment schemes

Prob. Scheme	Average Probability	Median Probability	# Intr with prob 0.5
BL	0.51	0.547	6,886
BH	0.477	0.496	5,896
DE	0.717	1	7,531
DG	0.39	0.25	4,799
SH	0.38	0.421	1,121
QI	0.97	0.99	11,658
AVG	0.574	0.671	7,866
EQ	0.99	0.99	11,883

Table 3. Correlation of different probability schemes. The p-values for all the correlation measurements were very significant (p-value 2e-16).

	BH	DE	DG	SH	QI
BL	0.923	0.655	0.633	0.626	0.371
	BH	0.672	0.644	0.665	0.416
		DE	0.718	0.847	0.238
			DG	0.68	0.466
				SH	0.274

3.2 Gene Ontology (GO) Similarity

As a second measure, we adopted the common notion that two interacting proteins are frequently involved in the same process and hence should have similar GO assignments [24]. The Gene Ontology terms are represented using a directed acyclic graph data structure in which an edge from term 'a' to term 'b' indicates that term 'b' is either a more specific functional type than term 'a', or is a part of term 'a'. As a result, terms that come deeper in the graph are more specific. Moreover, specific terms also have less number of proteins assigned to them. Hence, we evaluated the size (number of proteins assigned to the term) of the deepest common GO term assignment (deepest common ancestor) shared between a pair of proteins that interact. The gene ontology annotations for yeast proteins were obtained from the July 5th, 2005 download from Saccharomyces Genome Database (SGD) [26] and the association between terms were obtained from the Gene Ontology consortium (http://www.geneontology.org/).

Table 4. Association of interaction probabilities with the size of the deepest common ancestor in the Gene Ontology assignments and mRNA expression correlation. Shaded cells indicate schemes which used similar GO annotation or mRNA expression profiles as an input to assigning interaction reliability. *p*-values for all the correlation measurements were very significant (*p*-value 2e-16). SC: Spearman Correlation; MI: Mutual Inforamtion; WA: Weighted Average.

Prob. Scheme	GO Annotation			Expression Correlation		
	SC	MI	WA	SC	MI	WA
BL	-0.42	0.16	5.85	0.185	0.0531	0.494
BH	-0.5	0.22	5.68	0.223	0.0626	0.503
DE	-0.385	0.07	5.91	0.016	0	0.481
DG	-0.49	0.17	5.62	0.185	0.041	0.511
SH	-0.47	0.157	5.71	0.05	0.012	0.492
QI	-0.444	0.013	6.34	0.337	0	0.481
AVG	-0.545	0.26	5.93	0.205	0.08	0.585
EQ	—	—	6.32	—	—	0.482

Table 4 shows the relationship between the size of the deepest common GO term and interaction probabilities for each scheme. The probabilities generated by BH have relatively high correlation with the GO term assignments. This result is not surprising since gene ontology assignments are taken as input to probability calculation in this scheme. Although QI has a relatively high Spearman correlation coefficient, its weighted average and mutual information values are the worst.

3.3 Presence of Conserved Interaction in Other Species (Interologs)

Presence of conserved interactions across species is believed to be associated with biologically meaningful interactions [27]. However, since most species' interaction networks are still incomplete, it is important not to skew the results of this analysis due to false-negatives. As our benchmark, we used yeast protein interactions that were conserved with measured *C. elegans* (worm) and *D. melanogaster* (fly) interactions obtained from the Database of Interacting Proteins (DIP). An interaction was considered conserved if the orthologs of the interacting proteins were also interacting. Putative orthologs were assigned based on sequence similarity computed using BLAST [28]. We evaluated the weighted average between the probability assignment for each yeast interaction and the number of conserved interactions across worm and fly (0, 1, or 2) and repeated the analysis for different BLAST E-value thresholds for homology assignments (Fig. 1). At higher E-value thresholds we observe that SH has the highest weighted average but has similar values to BL and BH at lower thresholds. QI has similar weighted averages to Equal, and both consistently have lower values than the remaining schemes.

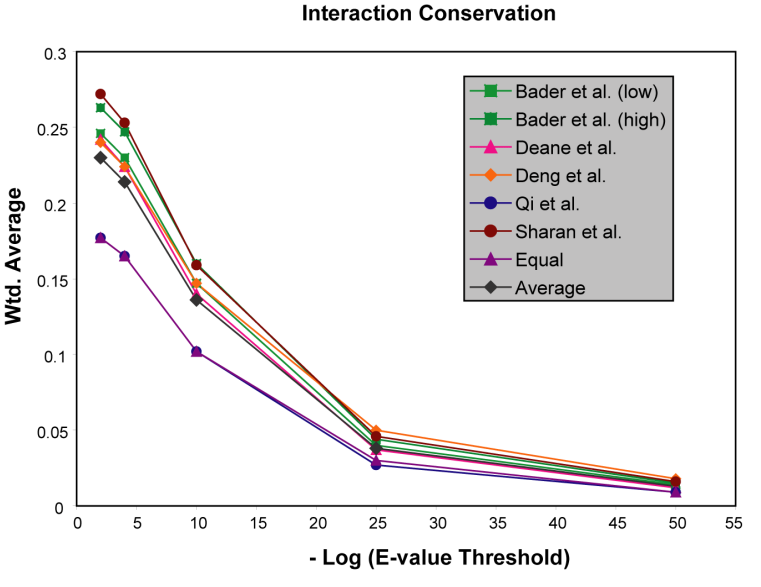

Fig. 1. Correlation of number of conserved interactions and probability assignments to interactions

3.4 Expression Correlation

Yeast expression data for ~790 conditions were obtained from the Stanford Microarray Database (SMD) [29]. For every pair of interacting proteins, we computed the Pearson correlation coefficient of expression. We then calculated the Spearman correlation, mutual information and weighted average between the expression correlation coefficient of interacting proteins and their corresponding probability assignments in the different schemes (see Table 4). We found significant association between expression correlations and probabilities in the case of BH, BL, QI and DG. This result is expected as these schemes, with the exception of BL, utilize expression similarity for interaction probability calculation. Surprisingly, DE probabilities showed very little correlation with expression, even though mRNA expression profiles were used as input in the prediction process. On the other hand, BL had a higher Spearman correlation and mutual information than SH, though they had very similar weighted averages and SH did not utilize expression data in the training phase.

3.5 Signals to Noise Ratio of Protein Complexes

Most cellular processes involve proteins that act together in pathways or complexes. Recently, several methods [19, 30-33] have been developed to ascertain the biologically meaningful complexes encoded within protein interaction networks. These methods search for complexes modeled as dense protein interaction sub-networks. Here, we applied a previously published algorithm [19] to discover complexes in the yeast network. We evaluated the resulting complexes using signal to noise ratio [34]. Signal to noise ratio (SNR) is a standard measure used in information theory and signal processing to assess data quality.

To compute SNR, a search for dense interaction complexes is initiated from each node (protein) and the highest scoring complex from each is reported. This yields a distribution of complex scores over all nodes in the network. A score distribution is also generated for 100 randomized networks which have identical degree distribution to the original network [35]. The randomized versions of interaction networks were generated by randomly reassigning the interactions, while maintaining the number of interactions per protein. SNR ratio is computed using these original and random score distributions (representing signal and noise, respectively) according to the standard formula [36] using the root-mean-square (rms):

$$\mathrm{SNR} = \log_{10} \frac{\mathrm{rms}(\text{original complex scores})}{\mathrm{rms}(\text{random complex scores})}, \quad \text{where } \mathrm{rms}(x) = \sqrt{\frac{1}{M}\sum_{i=1}^{M} x_i^2}$$

As the SNR is independent of the number of complexes reported, we can directly compare its value across the different probability schemes (Table 5). Here, DE and EQ probabilities have low SNR, while SH and DG have the highest SNR values.

Table 5. Associations of conservation rate coherency scores and SNR with interaction probabilities. SC: Spearman Correlation. Note that conservation scores based on weighted averages and mutual information were omited as they were similar across the different weighting schemes.

Prob. Scheme	Conservation Coherency (SC)	SNR
BL	-0.09	0.734
BH	-0.104	0.735
DE	-0.113	0.537
DG	-0.141	0.95
SH	-0.126	0.742
QI	-0.12	0.72
AVG	-0.132	0.73
EQ	—	0.657

Table 6. Ranking of the probability schemes in the five measures used for assessing their quality. Schemes with rank 1 perform the best. SC: Spearman Correlation; WA: Weighted Average; SNR: Signal to Noise Ratio. The shaded boxes indicate the measures used as input for the corresponsing probability scheme.

Probability Scheme	Gene Ontology (SC/WA)	Interaction Conservation (WA)	Gene Expression (SC/WA)	SNR	Conservation Coherency (SC)	Average Rank
Bader *et al.* (low)	6 / 4	3	4 / 4	4	7	4.14
Bader *et al.* (high)	2 / 2	2	2 / 3	3	6	3.66
Deane *et al.*	7 / 5	4	6 / 6	8	5	5.8
Deng *et al.*	3 / 1	4	4 / 2	1	1	2
Sharan *et al.*	4 / 3	1	5 / 5	2	3	3.28
Qi *et al.*	5 / 8	6	1 / 6	6	4	5.33
Average	1 / 6	5	3 / 1	5	2	3.28
Equal	- / 7	6	- / 6	7	-	6.5

3.6 Evolutionary Conservation

Interacting proteins show a clear preference to be conserved as a pair, indicating a selection pressure on the interaction links between proteins [20]. For every pair of interacting proteins, we computed the conservation rate coherency score as the absolute value of the difference between the evolutionary rates of the two corresponding genes. Low scores indicate highly coherent conservation rates. Evolutionary rates were obtained from Fraser *et al*. [21], estimated using nucleotide substitution rates. We then calculated the Spearman correlation between the conservation rate coherency scores of

interacting proteins and their corresponding probability assignments in the different schemes (see Table 6). For all probability assignment schemes we obtained a statistically significant negative correlation (p-value < 0.05) between the conservation rate discrepancy scores and the corresponding probabilities, indicating that proteins with high probability interactions tend to have similar conservation rates. The highest correlation (in absolute value) was obtained for DG.

4 Discussion

In summary, we have compared six of the available schemes that assign confidence scores to yeast interactions with each other and also with a uniform scheme. Table 6 gives the relative ranking of these schemes over the five measures used to assess their reliability.

Firstly, we find that EQ almost always ranks the lowest, suggesting that utilizing a probability scheme is always more beneficial than considering all observed interactions to be true. Secondly, QI has comparable ranks to other schemes when considering Spearman correlation coefficient, but generally has very low ranks when considering weighted average.

We conjecture that this trend is influenced by the relatively small standard deviation in the estimated probabilities in that scheme which assigns high probabilities to all interactions with (with 11447 interactions above 0.9) Thirdly, Deane *et al.* is the only scheme which assigns reliabilities to a set of interactions rather than individual interactions and generally performs poorly compared to other interactions schemes (Table 6). This suggests that probability schemes assessing the quality of each interaction by itself are more reliable.

We calculated the average ranks for each probability assignment schemes. To avoid circularity, the average ranks were computed by considering only those measures which were not used as input for the scheme in question. Overall, Deng *et al.* performs the best and Sharan *et al.* and the average scheme follow it as a close second.

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Appendix

Table A1. Spearman correlation of different measurement schemes. The p-values for the correlation measurements were very significant (p-value 2e-16).

	Interaction Conservation	**Conservation Coherency**	**Expression Correlation**
Gene Ontology	-0.14	0.14	-0.287
	Interaction Conservation	-0.08	0.058
		Conservation Coherency	-0.11