Protein domain connectivity and essentiality

L. da F. Costa, F. A. Rodrigues and G. Travieso
Instituto de Física de São Carlos, Universidade de São Paulo, PO Box 369, 13560-970, São Carlos, SP, Brazil

Protein-protein interactions can be properly modeled as scale-free complex networks, while the lethality of proteins has been correlated with the node degrees, therefore defining a lethality-centrality rule. In this work we revisit this relevant problem by focusing attention not on proteins as a whole, but on their functional domains, which are ultimately responsible for their binding potential. Four networks are considered: the original protein-protein interaction network, its randomized version, and two domain networks assuming different lethality hypotheses. By using formal statistical analysis, we show that the correlation between connectivity and essentiality is higher for domains than for proteins.

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A great deal of the functionality of proteins stems from their ability to dock, i.e. to connect. Such dockings are highly specific and depend on geometrical and field compatibilities between the involved proteins. More specifically, the docking sites of a protein are largely defined by the presence of specific domains, i.e. portions of aminoacid sequences along the protein primary backbone. Given that protein-protein interactions involve physical interactions between protein domains, domain-domain interaction information can be particularly useful for validating, annotating, and even predicting protein interactions. The subject of protein domain interaction has been covered in previous investigations.

Protein-protein interaction networks are obtained by representing each protein as a node and each possible docking between pairs of proteins as edges linking the respective nodes. Domain-domain interaction networks are constructed considering protein complexes, Rosetta Stone sequences, and by using protein interaction networks. The current work considers the last approach, taking into account domain subnetworks contained in protein-protein interaction networks. This method allows not only the direct visualization of the coexistence of domains and proteins, naturally providing for the multiplicity of domains, but also the objective quantification of interactions between domains.

Given a network, the degree of a specific node is defined as the number of connections between that node and the remainder of the network. This frequently used measurement can be generalized to express the connectivity not of a single node, but of a whole subnetwork contained in the original network. Subnetworks can be obtained by selecting a subset of nodes from the original network as a whole, but on their functional domains, which are ultimately responsible for their binding potential. The degree of a subnetwork is then defined as the number of connections between its nodes and the remainder of the network nodes, not taking into account the connections internal to the subnetwork. By quantifying the number of interactions between the subnetwork and the overall structure, the subnetwork degree provides a valuable indication about the role and importance of each subnetwork.

One particularly interesting way to define a subnetwork is by selecting among the nodes in the original network those that exhibit some specific feature. Considering a protein-protein interaction network, a subnetwork can be obtained by selecting those nodes that contain one or more instances of a specific protein domain. Note that such a subnetwork is embedded within the original protein-protein interaction network. A whole collection of subnetworks can then be obtained, one for each considered domain, and valuable insights about the importance and role of the domains can be inferred by using the concepts of subnetwork degree and subnetwork hubs. We applied such concepts to the Saccharomyces cerevisiae protein-protein interaction networks using the non-redundant database of interacting proteins by Sprinzak et al., which contains a large collection of multiple sequence alignments and profile hidden Markov models (HMM) covering the majority of protein domains, yielding a total of 1,424 domains. Figure 1 shows part of such a network, where four domain subnetworks are identified in black circles, white squares, black squares and black diamonds.

In order to investigate the relationship between domain connectivity and lethality, it is necessary to extend the concept of essentiality to domains. However, as there is no consensus about domain lethality in the scientific literature, we suggest the two following hypotheses:

1. Domain lethality in a weak sense: a domain is lethal if it appears in a lethal protein.
2. Domain lethality in a strong sense: a domain is lethal if it appears in a single-domain lethal protein.

The first definition is considered weak because a lethal domain can appear in lethal and viable proteins simultaneously. However, that assumption is still potentially interesting because co-occurring domains are more likely to exhibit similar function or localization than domain in separate proteins, which suggests that lethal proteins may involve uniformly lethal domains. The second
hypothesis, on the other hand, is considered strong because if the domain is the only one in a lethal protein, it must be responsible for the protein's essential function. When working with the first assumption, the whole protein interaction network is studied; for the second assumption, only the subnetwork formed by proteins with a single domain is considered. It is important to note that the two lethality situations above are just hypotheses to be checked against the experimental results reported by Jeong et al. concerning protein-protein interaction networks. In other words, eventual identification of high correlation between degree and lethality for one of those hypotheses could be understood as supporting that respective assumption due to the centrality-lethality rule, which is widely believed to reflect the special importance of hubs in organizing the network, and the biological significance of network architectures, a key notion in systems biology. Figure 2 shows the histogram of the cumulative protein degree and domain subnetworks degrees in both weak and strong senses. The cumulative degree distribution for all networks follows a power law with an exponential cut-off (finite size effect) described by $P(k) \approx (k + k_0)^\gamma e^{-(k+k_0)/k_c}$. The values of $k_c$ and $\gamma$ obtained from the cumulative distribution for proteins and domains are presented in Table I.

Figure 3 shows the relationship between degree and essentiality for the protein and domain networks. The lethality of proteins was determined using the MIPS database and the number of lethal protein, $N_L$, for the considered networks is shown in Table I. The abscissa represents the node degree $k$ of proteins or domains limited by the cut-off (see Figure 2) whose values are presented in Table I while the ordinate axis expresses the fraction of lethal proteins or domains among the ones with degree $k$. In order to determine the correlation between the fraction of lethal proteins/domains and their degree, we estimated the Pearson correlation coefficient $r$, which measures the strength of linear relationship between two variables, and the Spearman rank correlation coefficient $\rho$, which is a nonparametric coefficient used in case of nonlinear relationships. Table I presents the values of correlations for proteins and domains, which indicates that the correlation between lethality and degree is larger for hypotheses (I) and (II) than for whole proteins. The statistical significance of the correlations was tested by applying the Fisher’s comparison correlation coefficient test. The comparison between the correlation coefficients of proteins and domains in weak sense results $p \leq 0.035$ for $r$ and $p \leq 0.001$ for $\rho$. The comparison of proteins with domains in weak sense yields $p \leq 0.015$ for $r$ and $p \leq 0.001$ for $\rho$. These results lead to the conclusion that the domains correlations in both weak and strong sense are significantly higher than the correlation obtained for proteins.

Since protein domains represent the basic evolutionary units that form proteins, it is not surprising that domains should play a fundamental role in the definition of proteins interaction and lethality. In this way, the obtained results indicate that the interactions between proteins may be defined at the domain level, with the importance of domains being associated to their functions. As hubs tend to be the most important nodes in networks, domains with larger number of connections should be particularly fundamental (essential) for network maintenance. Indeed, domains with a high number of connections act as interconnecting pathways in the net-
A network (also called backbones) which, when removed, imply substantial network diameter increasal (as discussed by Jeong et al.\textsuperscript{9}). The special importance of domain essentiality can be readily inferred by inspecting the results presented in Figure\textsuperscript{3} and Table\textsuperscript{II}. Further, lethal domains are more likely to be hubs than lethal proteins. In other words, both hypotheses about domain lethality have been supported by the experimental results, with the strong sense hypothesis resulting more definite than the weak sense counterpart.

In order to verify whether the distribution of domains among proteins influences the domains connectivity, we randomized the protein positions along the network while maintaining the network structure, which was done by permutations of the proteins assigned to the nodes. Thus, for 100 randomized network versions (see in Figure 3(d)), the correlations obtained are close to zero (see Table II), confirming that the relation between the connectivity and lethality is unlikely to be a spurious effect.

The results presented here suggest a novel fundamental relationship between protein and domain interaction which has several implications for future works, as validating, annotating, and even predicting protein interactions and lethality. Also, our results can be used as a pre-investigation to obtain experimental data about domain interaction and lethality.

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17. The domain degree is normalized by the number of proteins present in the subgraph of the respective domain so as to avoid artificially high degree otherwise induced by more abundant domains, which would bias the results.