Specificity and stability in topology of protein networks

Sergei Maslov[1] and **Kim Sneppen**[2]
[1] *Department of Physics, Brookhaven National Laboratory, Upton, New York 11973, USA*
[2] *Department of Physics, Norwegian University of Science and Technology, N-7491 Trondheim, Norway.*

Abstract:
Molecular networks guide the biochemistry of a living cell on multiple levels: its metabolic and signalling pathways are shaped by the network of interacting proteins, whose production, in turn, is controlled by the genetic regulatory network. To address topological properties of these two networks we quantify correlations between connectivities of interacting nodes and compare them to a null model of a network, in which al links were randomly rewired. We find that for both interaction and regulatory networks, links between highly connected proteins are systematically suppressed, while those between a highly-connected and low-connected pairs of proteins are favored. This effect decreases the likelihood of cross talk between different functional modules of the cell, and increases the overall robustness of a network by localizing effects of deleterious perturbations.

With the growth of experimental information about basic biochemical mechanisms of life, molecular networks operating in living cells are becoming better defined. Direct physical interactions between pairs of proteins form one such network. It serves as a backbone for functional and structural relationships among its nodes and defines pathways for the propagation of various signals such as phosphorylation and allosteric regulation of proteins. The information about specific binding of proteins to each other has recently grown by an unprecedented amount as a result of high throughput two-hybrid experiments [1, 2]. The production and degradation of proteins participating in the interaction network is controlled by the genetic regulatory network of the cell formed by all pairs of proteins in which the first protein directly regulates the abundance of the second. The majority of known cases of such regulation happens at the level of transcription, in which a transcription fac-

tor positively or negatively regulates the RNA transcription of the controlled protein. The large scale structure of both these networks is characterized by a high degree of interconnectedness, where most pairs of nodes are linked to each other by at least one path. One may wonder how such a heavily intertwined and mutually dependent dynamical system can perform multiple functional tasks, and remain stable against deleterious perturbations.

We analyzed the topological properties of interaction and transcription regulatory networks in yeast *Saccharomyces cerevisiae*, which at present is perhaps the best characterized model organism. The interaction network used in this work consists of 4549 physical interactions between 3278 yeast proteins as measured in the most comprehensive two-hybrid screen of yeast proteins [2], while the genetic regulatory network is formed by 1289 directed positive or negative direct transcriptional regulations within a set of 682 proteins as listed in the YPD database [3]. The protein interaction network is a representative of the broad class of scale-free networks [4, 5, 6] in which the number of nodes with a given number of neighbors (connectivity) K scales as a power law $\propto 1/K^{\gamma}$. In our case the histogram of connectivities can be fitted by a power law with $\gamma = 2.5 \pm 0.3$ for K ranging from 2 to about 100 [7, 8]. A small part of the protein interaction network, formed by proteins known to be localized in the nucleus and to interact with at least one other nuclear protein, was visualized (Fig. 1). One striking feature of this graph is the abundance of highly connected proteins that are mostly connected to those with low connectivity, and thus well separated from each other.

To test for correlations in connectivities of nodes for each of the above two networks we calculated the likelihood $P(K_0, K_1)$ that two proteins with connectivities K_0 and K_1 are connected to each other by a link and compared it to the same quantity $P_r(K_0, K_1)$ measured in a randomized version of the same network. In this "null model" network all proteins have exactly the same connectivity as in the original one, while the choice of their interaction partners is totally random. The transcription regulatory network is naturally directed, while the network of physical interactions among proteins in principle lacks directionality. However, for poorly understood reasons the two-hybrid experimental data have a significant asymmetry between baits and preys, with bait hybrids being more likely to be highly connected than their prey counterparts. This can be seen e.g. in the fact that average connectivity of baits with at least one interaction partner is close to 3, whereas

the same quantity measured for preys is only 1.8. Since each reported interaction involves one bait and one prey protein, this asymmetry needs to be taken into account when constructing an uncorrelated "null" model for the interaction network. For this purpose in our randomization procedure we would treat the two-hybrid data as a directed network with an arrow on each edge pointing out from bait to prey hybrid. Randomized versions of these two networks were constructed by randomly reshuffling links, while keeping the in- and out-degree of each node constant. A convenient numerical algorithm performing such randomization consists of first randomly selecting a pair of directed edges A→B and C→D The two edges are then rewired in such a way that A becomes connected to D, while C to B. However, in case if one or both of these new links already exist in the network this step is aborted and a new pair of edges is selected. This last restriction prevents the appearance of multiple edges connecting the same pair of nodes. A repeated application of the above rewiring step leads to a randomized version of the original network. Multiple sampling of randomized networks allowed us to calculate both the average expectation and the standard deviation for any particular property of the random network.

Correlations in connectivities manifest themselves as systematic deviations of the ratio $P(K_0, K_1)/P_r(K_0, K_1)$ from 1. We calculated this ratio for interaction (Fig. 2A) and regulatory (Fig. 2B) networks, with K_0 and K_1 being the total number of interaction partners of two interacting proteins (for the interaction network), and out- and in-degrees of two nodes connected by a directed edge 0→1 (for the regulatory network). Thus by the very construction $P(K_0, K_1)$ is symmetric for the physical interaction network but not for the regulatory network. We also estimated the statistical significance $Z(K_0, K_1)$ of the above deviations in the interaction (Fig. 2C) and regulatory (Fig. 2D) networks, by dividing each observed deviation from the null model by the standard deviation in multiple realizations of a randomized network. The combination of these two plots reveals the regions on the $K_0 - K_1$ plane, where connections between proteins in the real network are significantly enhanced or suppressed, compared to the null model. In particular red regions in the upper left and the lower right corners reflect the tendency of highly connected nodes (hubs) to associate with nodes of low connectivity, while the blue/green region in the upper right corner reflects the reduced likelihood that two hub centers are directly linked to each other. One should also note a prominent feature on the diagonal of the Fig. 2A and

2C corresponding to an enhanced affinity of proteins with between 4 and 9 interaction partners to physically interact with each other. This feature can be tentatively attributed to the tendency of members of multi-protein complexes to interact with other proteins from the same complex. The above range of connectivities thus correspond to a typical number of direct interaction partners of a protein in a complex. When we checked for interactions between proteins in this range of connectivities we found 39 pairs of interacting proteins to belong to the same complex in a recent high throughput study [9], which is 4 times more than one would expect to find by pure chance alone.

To further quantify and compare correlation patterns in interaction and regulatory networks we calculated the average connectivity $\langle K_1 \rangle$ of nearest neighbors of a node, as a function of its own connectivity K_0 (Fig. 3A). In order to simplify the comparison between two networks here we characterize each node in the regulatory network by its total number of neighbors $K = K_{in} + K_{out}$. For both interaction and regulatory networks the average connectivity $\langle K_1 \rangle$ shows a gradual decline with K_0, which can be fitted with a power law $\langle K_1 \rangle \propto 1/K_0^{0.6\pm0.1}$ over approximately two decades. This observation gives an additional credit to the affinity between correlation patterns in these two protein networks visible in Fig. 2. It was recently found [10] that the internet, defined as the set of interconnected routers, in addition to a scale-free distribution of node connectivities similar to the protein interaction network, is characterized by the same correlation pattern between connectivities of neighboring nodes: $\langle K_1 \rangle \propto 1/K_0^{0.5}$. This extends by one step an intriguing similarity in the topology of these networks of completely different nature.

For the scale-free physical interaction network we also plotted the probability distribution of the nearest neighbor connectivity K_1, measured separately for nodes with small connectivity $K_0 \leq 3$, and for those with large connectivity $K_0 \geq 100$ (Fig. 3B). In the absence of correlations this conditional probability does not depend on K_0, and is proportional to $K_1/K_1^{\gamma} \sim 1/K_1^{1.5}$. This uncorrelated form holds approximately true for neighbors of a protein with low connectivity. It is only violated at the far tail of the distribution due to an excess likelihood of it being connected to a protein with very high connectivity, as was mentioned above. On the other hand, the distribution of connectivities K_1 of neighbors of highly connected proteins scales as $\propto 1/K_1^{2.5}$ and thus differs from that of lowly connected ones by a factor of $1/K_1$.

When analyzing molecular networks one should consider possible sources of errors in the underlying data. Two-hybrid experiments give rise to false positives of two kinds. In one case the interaction between proteins is real but it never happens in the course of the normal life cycle of the cell due to spatial or temporal separation of participating proteins. In another case an indirect physical interaction is mediated by one or more unknown proteins localized in the yeast nucleus. Reversely, in a high throughput two-hybrid screens one should expect a sizeable number of false negatives. Primarily a binding may not be observed if the conformation of the bait or prey heterodimer blocks relevant interaction sites or if the corresponding heterodimer altogether fails to fold properly. Secondly, 391 proteins out of the potential 5671 baits in [2] were not tested as possible bait hybrids because they were found to activate transcription of the reporter gene in the absence of any prey proteins.

Unlike for the interaction network, our data for the genetic regulatory network do not come from a single large scale project. Instead, they represent a collection of numerous experiments performed by different experimental techniques in different labs. Therefore, it is not feasible even to list possible sources of errors present in such a diverse data set. In particular one should worry about a hidden anthropomorphic factor present in such a network: some proteins just constitute more attractive subjects of research and are, therefore, relatively better studied than others. One should also note that the transcription regulation network is only a subset of a larger genetic regulatory network, which in addition to transcriptional regulation includes translational regulation, RNA editing, etc. An encouraging sign was that when we separately analyzed the set representing the current knowledge [3] about this later more complete network, consisting of 1750 genetic regulations among 848 proteins we reproduced all of our empirical results for the transcriptional network.

The observed suppression of connections between nearest neighbors of highly connected proteins is consistent with compartmentalization and modularity characteristic of control of many cellular processes [11]. In fact, it suggests the picture of functional modules of the cell organized around individual hubs. To further test the extent of modularity of hubs and their immediate neighborhood in each network we selected 15 highest connected nodes. To provide an unbiased sample of hubs from the point of view of in and out connectivity half of those nodes were selected as the highest out-degree hubs (8 baits with $K_{bait} \geq 90$ for the interaction network and 7 nodes with

$K_{out} \geq 34$ for the regulatory network), while half were the highest in-degree hubs (7 preys with $K_{prey} \geq 20$ for the interaction network and 8 nodes with $K_{out} \geq 8$ for the regulatory network). In agreement with the correlation properties described above, direct connections between hubs were significantly suppressed. In the interaction network we observed 20 links between different hubs in this group, which is significantly below 56 ± 7.5 links in the randomized network. In the transcription regulatory network there were 16 links between hubs in real network as opposed to 35 ± 6.5 in its randomized version. Not only direct links between hubs are suppressed in both studied networks, but hubs also tend to share fewer of their neighbors with other hubs, thereby extending their isolation to the level of next-nearest neighbor connections. The total number of paths of length 2 between the set of 15 hubs in the interaction network is equal to 418, whereas in the null model we measured this number to be 653 ± 56. Similarly, for the transcriptional network the number of paths of length 2 is equal to 186 in the real network, whereas from the null model one expects it to be 262 ± 30. Since the number of paths of length 2 between a pair of proteins is equal to the number of their common interaction partners one concludes that both the hub node itself and its immediate surroundings tend to separate from other hubs, reinforcing the picture of functional modules clustered around individual hubs.

A further implication of the observed correlation is in the suppression of the propagation of deleterious perturbations over the network. It is reasonable to assume that certain perturbations such as e.g. significant changes in the concentration of a given protein (including its vanishing altogether in a null-mutant cell) with a ceratin probability can affect its first, second, and sometimes even more distant neighbors in the corresponding network. While the number of immediate neighbors of a node is by definition equal to its own connectivity K_0, the average number of its second neighbors, given by $K_0\langle(K_1 - 1)\rangle_{K_0}$, is sensitive to correlation patterns of the network. Since highly connected nodes serve as powerful amplifiers for the propagation of deleterious perturbations it is especially important to suppress this propagation beyond their immediate neighbors. It was argued that scale-free networks in general are very vulnerable to attacks aimed at highly connected nodes [12, 13]. The anticorrelation presented above implies a reduced branching ratio around these nodes and thus provides a certain degree of protection against such attacks. This may be the reason why the correlation between the connectivity of a given protein and the lethality of the mutant cell lacking

this protein is not particularly strong [8].

It is feasible that molecular networks in a living cell have organized themselves in an interaction pattern that is both robust and specific. Topologically the specificity of different functional modules can be enhanced by limiting interactions between hubs and suppressing the average connectivity of their neighbors. We have seen that such correlation pattern appears in a similar way in two different layers of molecular networks in yeast, and thus presumably is a universal feature of all molecular networks operating in living cells.

References

[1] P. Uetz, *et al. Nature* **403**, 623 (2000).

[2] T. Ito, *et al.* interactome. *Proc. Natl. Acad. Sci. USA* **98**, 4569 (2001).

[3] M. C. Costanzo, *et al. Nucleic Acids Research* **29** 75

[4] A.-L. Barabasi and R. Albert, *Science*, **286**, 509 (1999).

[5] A. Broder, *et al.*, *Computer Networks*, **33**, 309-320 (2000).

[6] H. Jeong, B. Tombor, R. Albert, Z. N. Oltvai, A.-L. Barabasi, *Nature*, **407**, 651 (2000); D.A. Fell, A. Wagner, *Nat Biotechnol.* **18** 1121 (2000).

[7] A. Wagner, *Mol. Biol. Evol.* **18**, 1283(2001).

[8] H. Jeong, S. Mason, A.-L. Barabasi, Z.N. Oltvai, *Nature* **411**, 41 (2001).

[9] A.-C. Gavin, *et al. Nature* **415**, 141 (2002).

[10] R. Pastor-Satorras, A. Vazquez, A. Vespignani, Phys. Rev. Lett. **87**, 258701 (2001).

[11] L.H. Hartwell, J.J. Hopfield, S. Leibler, and A.W. Murray, *Nature* **402** (6761 Suppl), C47 (1999).

[12] R. Albert, H. Jeong, A.-L. Barabasi, *Nature* **406**, 378 (2000).

[13] B. Vogelstein, D. Lane, and A.J. Levine, *Nature* **408**, 307-310 (2000).

[14] Supported in part by the National Science Foundation under Grant No. PHY99-07949. Work at Brookhaven National Laboratory was carried out under Contract No. DE-AC02-98CH10886, Division of Material Science, U.S. Department of Energy. We thank the Institute for Theoretical Physics at the University of California at Santa Barbara, Nordita, and the University of Tokyo (for S.M.) for hospitality, K. Eriksen for valuable comments on the manuscript, T. Hwa for suggesting the subject of two-hybrid experiments, and T. Ito for providing the infomation on limitations of his experimental techniques.

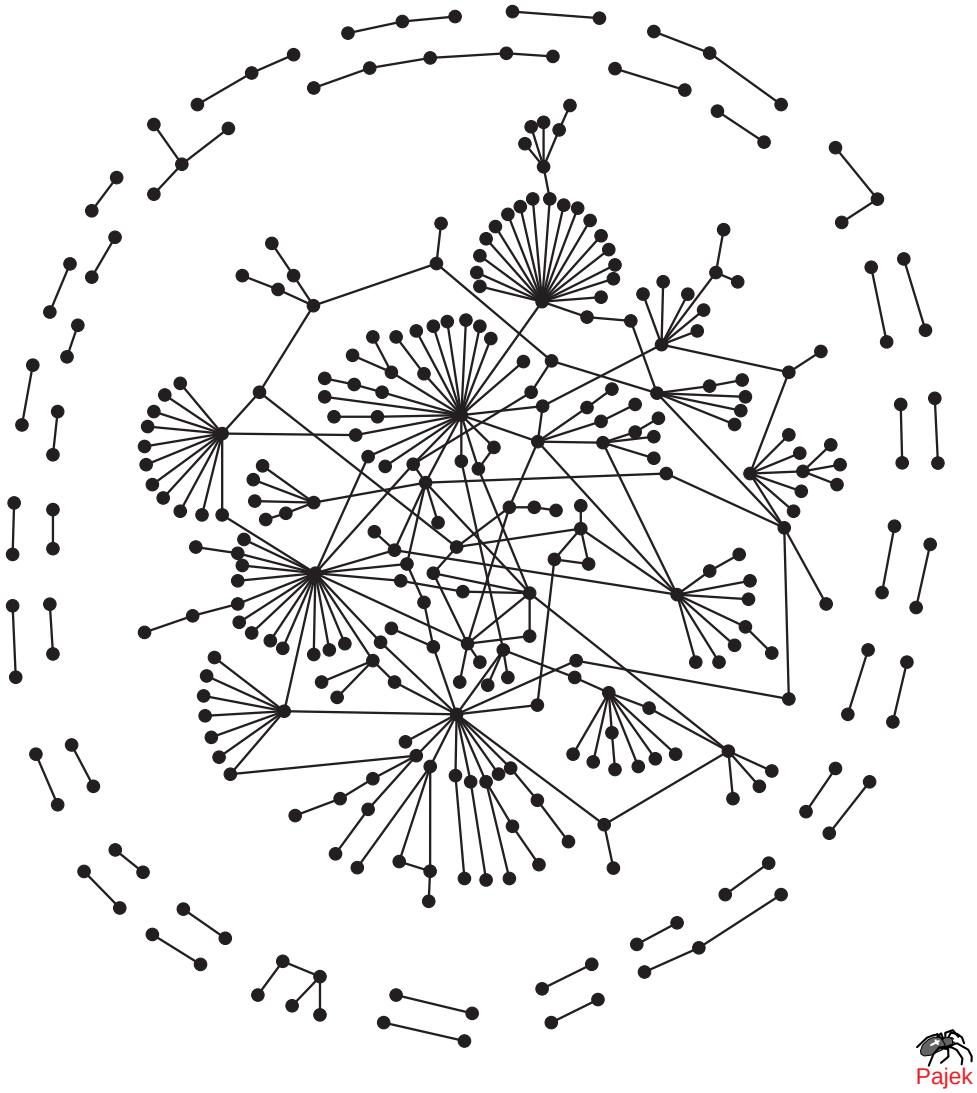
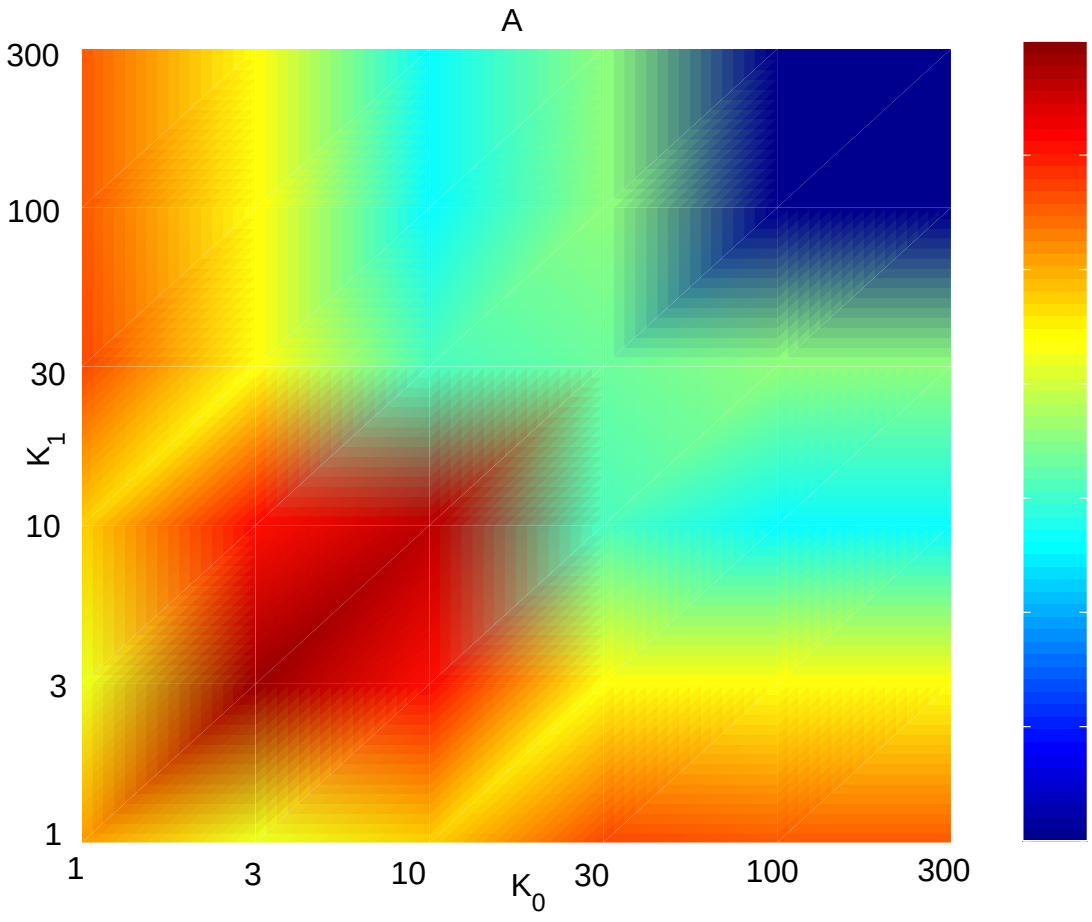
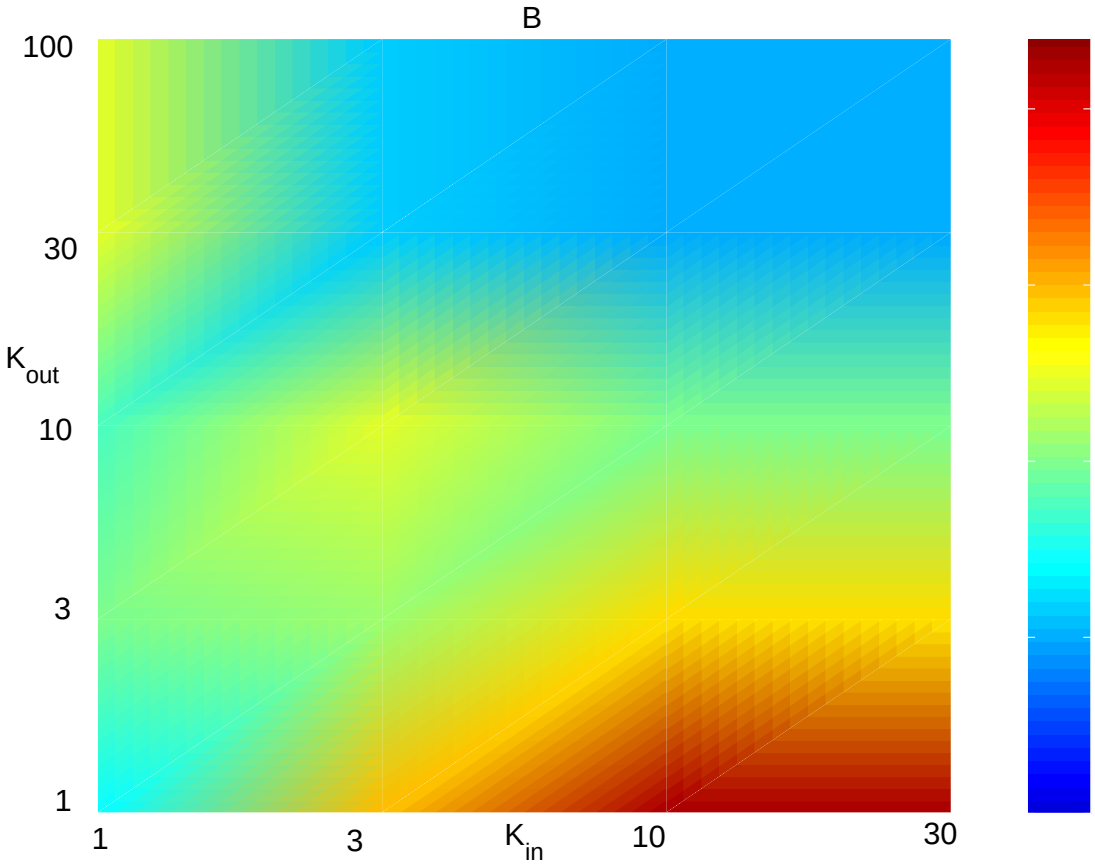
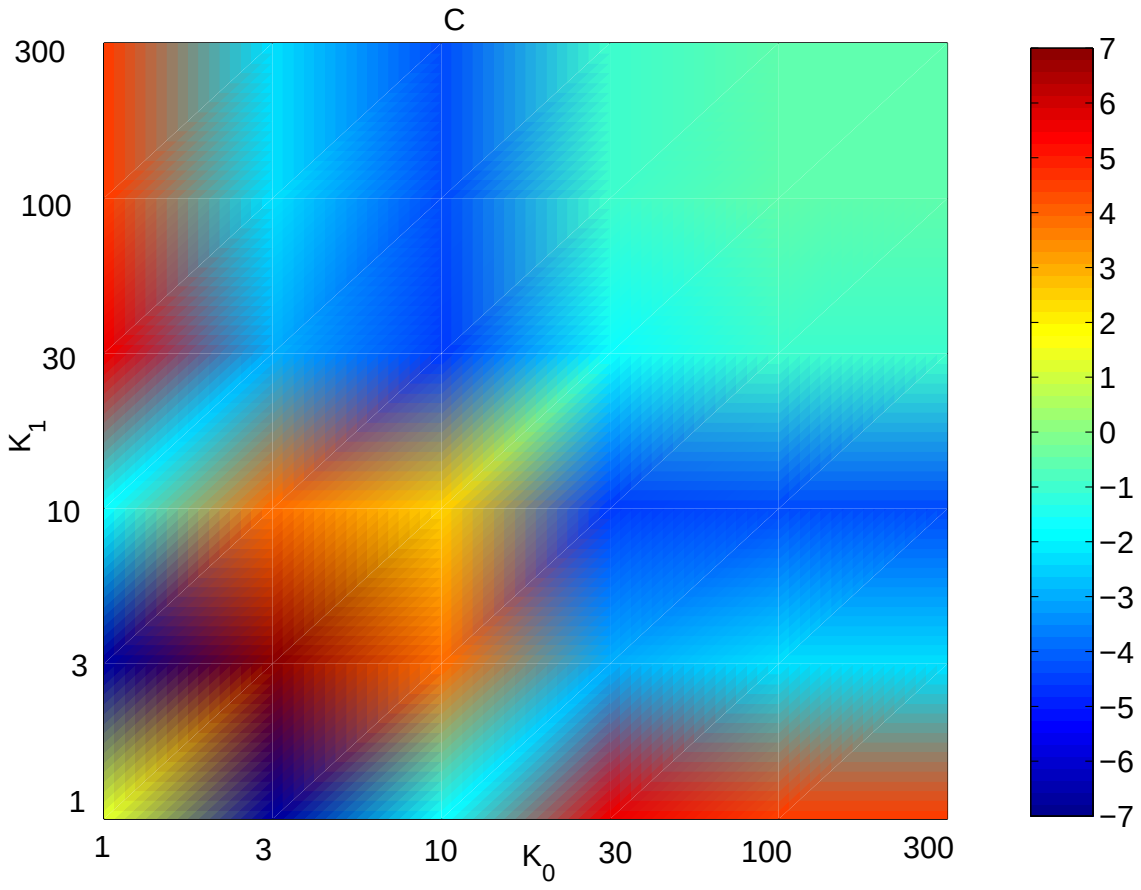


Figure 1: Network of physical interactions between nuclear proteins. Here we show the part of the network reported in [2], consisting of all proteins that are known to be localized in the yeast nucleus [3], and which interact with at least one other protein in the nucleus. This subset consists of 318 interactions between 329 proteins. Note that most neighbors of highly connected nodes have rather low connectivity.


A
300
100
30
10
3
1
K1
1
3
10
30
100
300
K0


B
100
30
10
3
1
K out
1
3
10
30
K in


C
300
100
30
10
3
1
K1
1
3
10
30
100
300
K0
7
6
5
4
3
2
1
0
−1
−2
−3
−4
−5
−6
−7

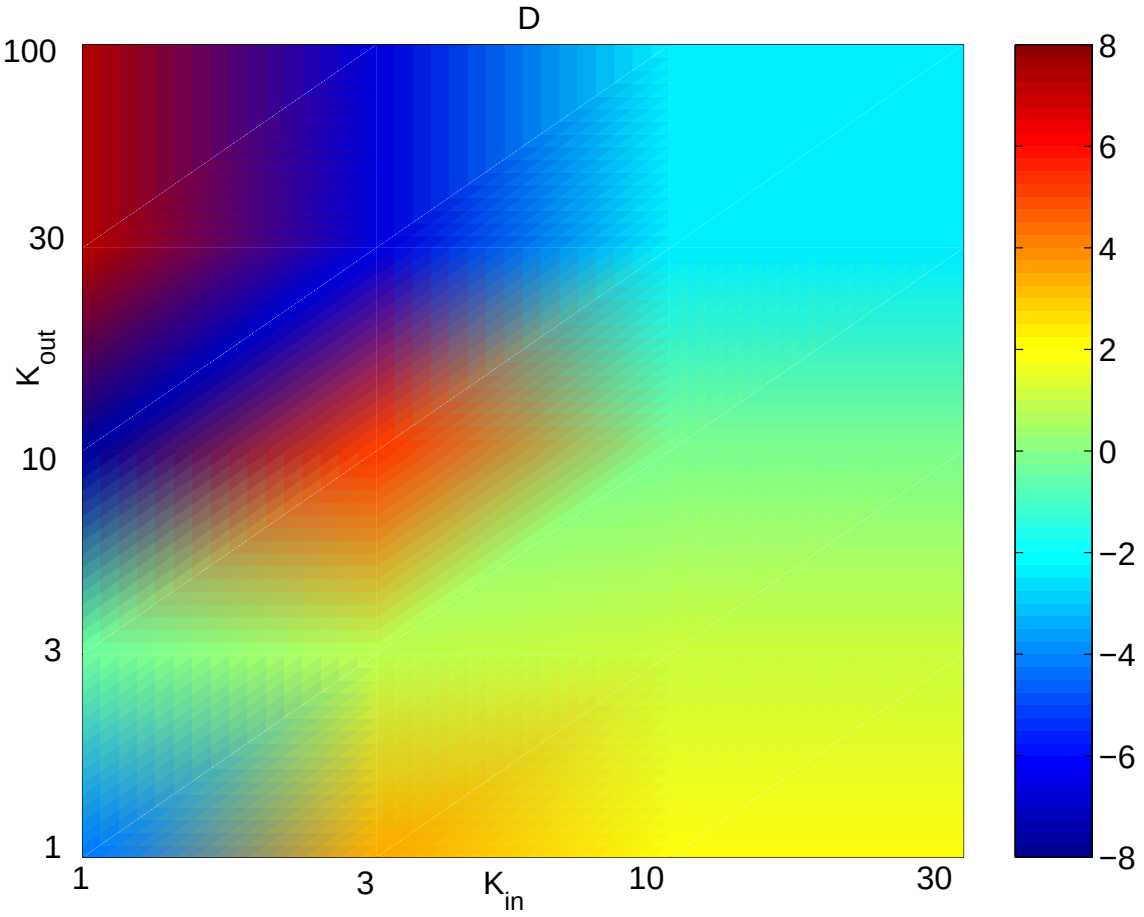
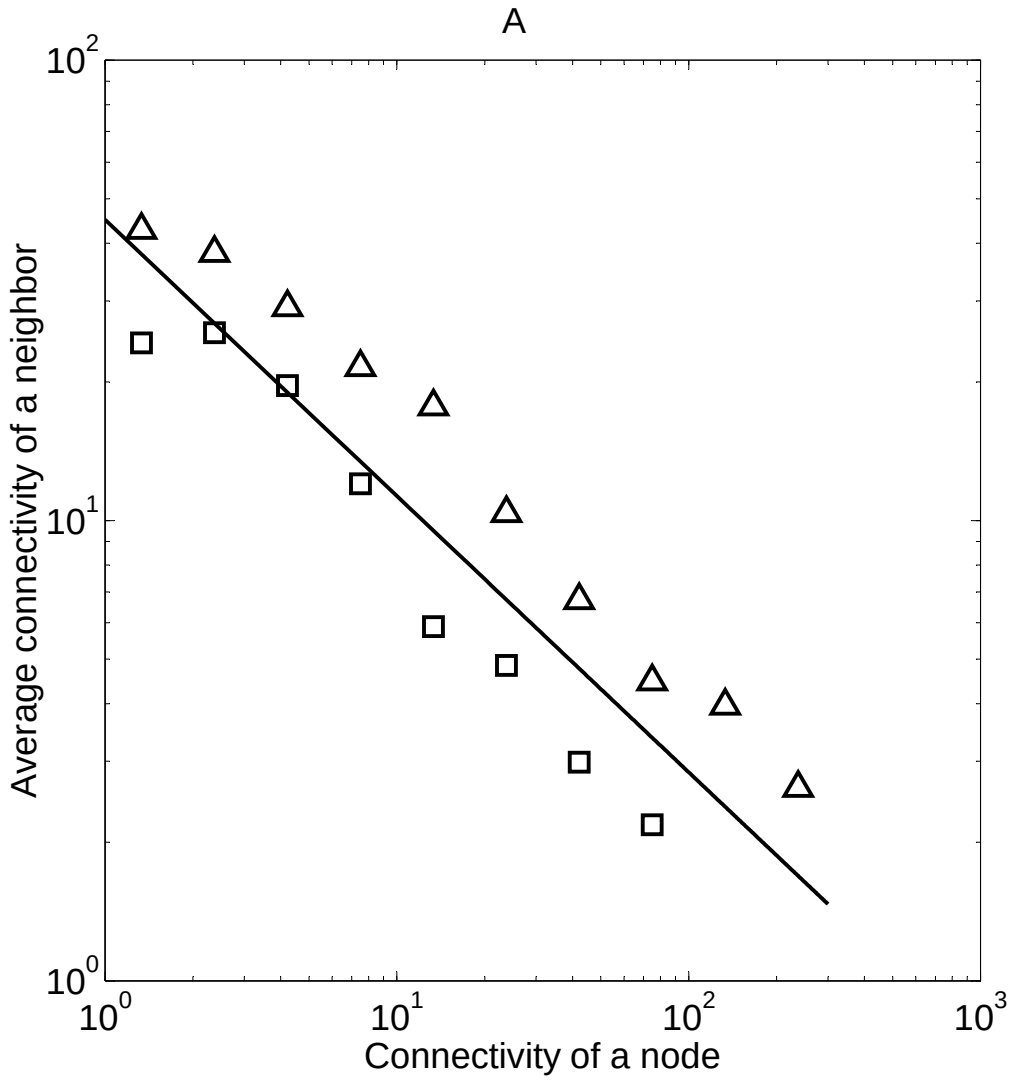

D
100
30
10
3
1
K_out
1
3
10
30
K_in
8
6
4
2
0
−2
−4
−6
−8

Figure 2: Correlation profiles of protein interaction and regulatory networks in yeast. (A) The ratio $P(K_0, K_1)/P_r(K_0, K_1)$, where $P(K_0, K_1)$ is the probability that a pair of proteins with total numbers of interaction partners given by K_0, K_1 correspondingly, directly interact with each other in the full set of [2], while $P_r(K_0, K_1)$ is the same probability in a randomized version of the same network. (B) The same as (A) but for a protein with the in-degree K_{in} to be regulated by that with the out-degrees K_{out} in the transcription regulatory network [3]. (C) Z-scores for connectivity correlations from (A): $Z(K_0, K_1) = (P(K_0, K_1) - P_r(K_0, K_1))/\sigma_r(K_0, K_1)$ where $\sigma_r(K_0, K_1)$ is the standard deviation of $P_r(K_0, K_1)$ in 1000 realizations of a randomized network. (D) As in (C) but for incoming and outgoing links in the the transcription regulatory network. To improve statistics the connectivities in all four panels of Fig. 2 were logarithmically binned into 2 bins per decade.


A
10^2
10^1
10^0
Average connectivity of a neighbor
10^0
10^1
10^2
10^3
Connectivity of a node

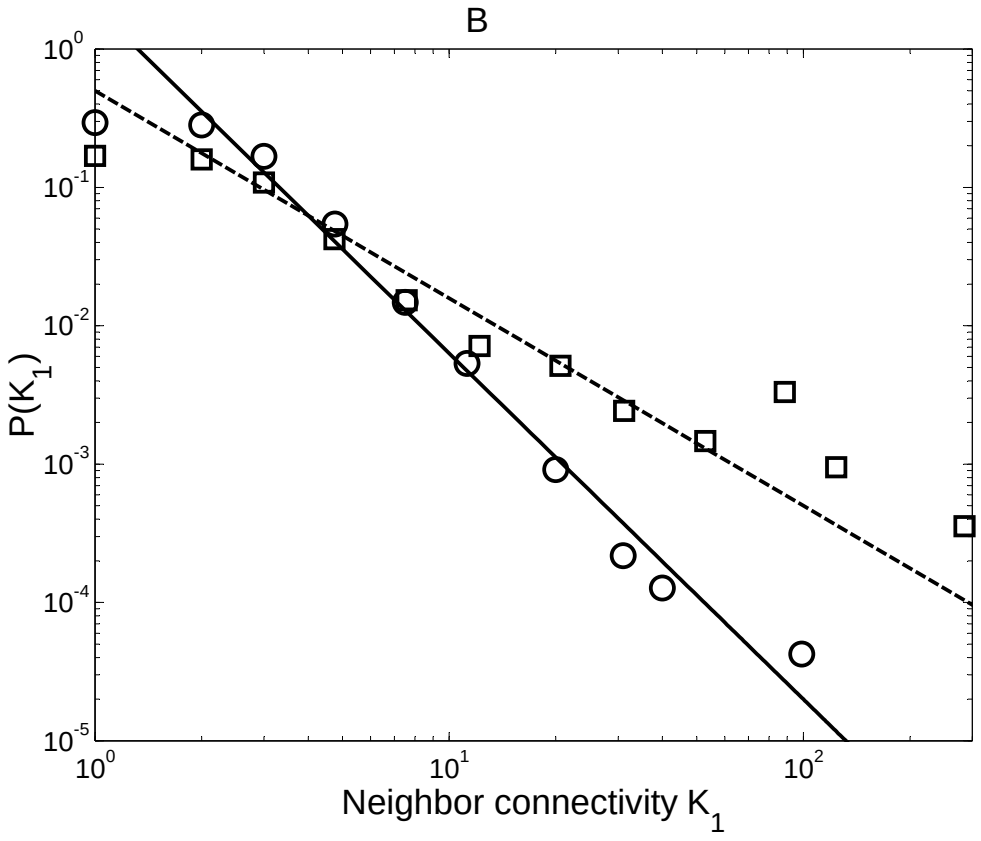

B
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
$P(K_1)$
10^0
10^1
10^2
Neighbor connectivity K_1

Figure 3: Correlations in connectivities of neighbors. (A) The average connectivity $\langle K_1 \rangle$ of nearest neighbors of proteins with the connectivity K_0 in the physical interaction network (triangles) and the regulatory network (squares). The solid line is a power law fit, $\propto 1/K_0^{0.6}$. (B) The probability distribution of connectivities K_1 in the physical interaction network calculated separately for neighbors of proteins with small connectivity $K_0 \leq 3$ (squares), and with large connectivity $K_0 \geq 100$ (circles). Lines are power laws $\propto 1/K_1^{1.5}$ (dashed) and $\propto 1/K_1^{2.5}$ (solid).