COMPUTATIONAL BIOLOGY

Network-Based Tools for the Identification of Novel Drug Targets

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In the past few years, network-based tools have become increasingly important in the identification of novel molecular targets for drug development. Systems-based approaches to predict signal transduction–related drug targets have developed into an especially promising field. Here, we summarize our studies, which indicate that modular bridges and overlaps of protein-protein interaction and signaling networks may be of key importance in future drug design. Inter-modular nodes are very efficient in mediating the transmission of perturbations between signaling modules and are important in network cooperation. The analysis of stress-induced rearrangements of the yeast interactome by the ModuLand modularization algorithm indicated that components of modular overlap are key players in cellular adaptation to stress. Signaling crosstalk was much more pronounced in humans than in *Caenorhabditis elegans* or *Drosophila melanogaster* in the SignaLink (http://www.SignaLink.org) database, a uniformly curated database of eight major signaling pathways. We also showed that signaling proteins that participate in multiple pathways included multiple established drug targets and drug target candidates. Lastly, we caution that the pervasive overlap of cellular network modules implies that wider use of multitarget drugs to partially inhibit multiple individual proteins will be necessary to modify specific cellular functions, because targeting single proteins for complete disruption usually affects multiple cellular functions with little specificity for a particular process. Tools for analyzing network topology and especially network dynamics have great potential to identify alternative sets of targets for developing multitarget drugs.

Presentation Notes

Slide 1: Science Signaling *logo*

The slideshow and notes for this presentation are provided by *Science Signaling* (http://www.sciencesignaling.org).

Slide 2: Network-based tools for the identification of novel drug targets

Despite considerable efforts and resources, the number of novel drug targets identified during the past decade has fallen behind expectations. This presentation summarizes several network-based approaches to aid drug target identification. Phosphoproteomes, kinomes, and signaling networks are especially powerful systems-based resources for predicting novel therapeutic targets (*1*–*5*). We show how different methods of analyzing network topology and dynamics may help drug discovery.

Slide 3: Advantages of the network approach

Real-world networks have many general properties, such as small-worldness (a concept first described by the Hungarian writer Frigyes Karinthy in 1929); scale-free type degree distribution, which is an increased prevalence of network hubs compared with random networks; hierarchical and nested topology; as well as a key role of links that are long-range, low-affinity, low-probability, and weak in terms of both information transfer and network stability (*6*–*10*). The surprisingly high number of patterns shared by various real-world networks endows the network approach with at least two special advantages for researchers. First, the investigator is able to judge the importance of his or her discovery by determining whether a finding has any of the generalities that appear in many molecular, technological, and social networks and ecosystems. Secondly, the network approach provides a route for the systematic translation of the original ideas into the context of other scientific fields that may differ linguistically from the researcher's field of expertise. This often solves creativity deadlocks, because the novel context may offer additional and even unexpected associations and solutions. Therefore, the network-based systematic approach offers both a gauge of importance and a boost to creativity.

Slide 4: Aging as an early warning signal of a critical phase transition: death

Sometimes insight into a system can arise from an unexpected source. Such a surprising system-based transfer of knowledge from one context to another came when I (P.C.) read the seminal review by Scheffer *et al*. (*11*) on the "Early warning signals of critical phase transitions," wherein the authors examined the common signs of pre−crisis periods in ecosystems, markets, and climate. In this review, three major warning signals were highlighted: (i) "slower recovery from perturbations," (ii) the "increased self-similarity of behavior," and (iii) the "increased variance of fluctuation patterns." Based on our earlier studies on aging (*12*, *13*), it was imminently clear to me that an aging organism shows the very same three signs of change. Thus, aging can be perceived as an early warning signal of a critical phase transition, wherein the phase transition is death. However, this sobering message also has a positive implication: Phase transitions of complex systems can be slowed down, postponed, or prevented by elements of independent and unpredictable behavior, such as stem cells (in the case of aging), top predators (in ecosystems), or the actions of market gurus (in economies). Thus, the good news is that the identification of interactome or signaling nodes with unpredictable behavior may offer novel molecular targets for anti-aging therapies.

Slide 5: Creative elements as possible network targets of anti-aging therapies

Networks can be characterized by a few distinct types of behavior. Most nodes are "problem solvers." These nodes are specialized to a certain task that they can complete (solve) with high efficiency. A few nodes are "problem distributors." These nodes have a large number of neighbors, meaning that they

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are hubs, and are specialized to the distribution of perturbations and signals. Both problem solvers and problem distributors have rather predictable behavior patterns. Nodes of the third type exhibit more exotic behavior. These "creative elements" are extremely dynamic, and, by continuous change in the structure of their links, sample practically the entire network. Creative elements also have rather few links at any given time, and many of these links connect the creative elements with hubs or other key nodes of modules and intermodular connections. Furthermore, the internal structure of creative elements (the underlying network of atoms, proteins, cells, or persons that constitute the creative node of the upper-level network of proteins, cells, persons, or society) is more flexible than that of problem solvers or distributors. In contrast to the other two types of nodes, the actions and outputs of creative elements are highly unpredictable (*14*–*16*). This high unpredictability makes the creative elements of protein-protein interaction networks, such as molecular chaperones, promising targets of anti-aging therapies (*10*, *12*, *13*). The three types of behaviors described above can be mixed in more complex networks, particularly those encountered in the real world.

Slide 6: Creative elements as dynamic bridges and overlaps of network modules

Creative elements occupy central positions in their networks and connect network regions that would otherwise be distant. Such positions bridge the "structural holes" of social networks described by Ronald S. Burt (*15*). The active centers and binding sites of proteins often occupy such positions in protein structure networks. As the complexity of the system increases, the mobility of individual creative elements expands so that these elements cover more and more of the network. (We are yet unable to judge whether the number of creative elements indeed increases with the level of complexity, as one would expect.) Creative elements are often found in regions of overlap between modules and so belong to a certain extent to two or more modules, or bridge two or more modules, meaning that their neighborhoods occupy a comparably large space in multiple modules (*14*–*16*). Unique and monopolistic intermodular positions have also been termed "bottlenecks" (*17*), because almost all information flowing through the network must pass through these key nodes. Creative elements may often behave as temporary bottlenecks, with other nodes later substituting for the creative element.

Slide 7: The interrelated network-set helping drug discovery

Drug discovery is facilitated when multiple networks describing the relationships of patients and their genetics, symptoms, diseases, therapies, drug targets, and drugs are considered. In most cases these networks are bipartite networks, wherein two separate groups of data are linked, such as patients being linked with symptoms, medications, therapies, or genetic background. Similarly, bipartite networks may be formed by linking a disease with the genes involved in the etiology of that disease, or by linking drugs with their targets or the therapies in which they are employed. On the slide, white arrows mark data sets for which bipartite networks have already been published, whereas black arrows denote some possibilities for future network analysis studies. All these bipartite networks can be visualized and analyzed as corresponding pairs of networks, wherein, for example, two drugs are linked if they have a common target, or two drug targets are linked if there is a drug that affects both (*18*, *19*). The large number of disease- and drug-related networks (*18*) will have a major impact on therapeutic approaches for targeting signaling pathways (*1*–*5*).

Slide 8: Examples of promising network positions for drug target candidates

Examination of the complex topology of protein-protein interaction networks and signaling networks highlights several key nodes that may be important for drug design. Hubs are nodes with more neighbors than the average for other nodes in the network. Hub connections are specific interactions that link hubs. Overlapping elements are nodes that belong to two or more network modules. Bridges are nodes that connect two network modules and play a substantial role in each module. All of these are promising drug target candidates, because targeting such nodes or links may influence many more nodes responsible for a cellular function that affects a particular disease (*20*). In our work, we have studied the properties of overlapping elements and bridges, both of which can be identified by their special positions in the structure of complex networks.

Slide 9: Turbine: A widely applicable algorithm for assessing network perturbation dynamics

The examination of network dynamics is of paramount importance for understanding network topology. Analysis of network dynamics also increases our understanding of cellular signaling. We have developed Turbine, a widely applicable, Matlab-compatible tool kit, to assess the propagation of perturbations through cellular networks, including interactomes and signalomes. This flow chart illustrates how the Turbine software (*21*, *22*), which is freely available at http://www.linkgroup.hu/Turbine.php, works. Turbine requires the list of network contacts (marked as "pajek," referring to one of the input format of the network data) and the perturbation model ("model.dll") as inputs. The program supplies a few perturbation models, such as the "communicating vessels" model, but may be extended by the addition of any other models, by multiple perturbations, or by models for the propagation and dissipation of perturbations. The program may simulate the perturbations of real-world networks that include 1 million nodes and 10 million links per gigabyte of free system memory. The output, marked as "mat," reports the level of perturbation at any network node at any time point. Numerical data can be converted to a visual form by using the Turbine viewer software ("Turbine GUI").

Slide 10: Modular overlaps and bridges are primary transmitters of network perturbations

As an example highlighting the importance of intermodular nodes (overlapping elements and bridges), we compared the propagation of a perturbation from a starting node in the module center of the modular scale-free benchmark network of Lancichinetti *et al*. (*23*) (left diagram) with that same perturbation initiated from a node that bridges two modules (right diagram) (*22*). The number of nodes affected by the perturbation after 600 iterations (shown as yellow, orange, and red dots) is much larger when the perturbation started from the bridge node (arrow in right diagram) than the center node (arrow in left diagram) in this sample network with relatively condensed modules. Intermodular nodes play a prominent role in signal transduction (*4*) and in the propagation of allosteric signals in protein structures (*24*). Recently we proposed that signaling through "cumulus-type" networks, which have limited overlap between modules and a more compact module structure, can be generally described by an energy-transfer mechanism, which has been observed between "independent dynamic segments of proteins" behaving as "discrete breathers," which are smaller parts of proteins that move independently of other protein segments (*16*, *24*). In "stratus-type" networks, in which modules overlap considerably, information transfer inside modules occurs

through multiple trajectories. These signaling trajectories converge at modular boundaries to a few bridging nodes. Bridging nodes, such as the intermodular node highlighted by the arrow in the right diagram, may have a key role in regulating signal transmission from one network module to another (*24*).

Slide 11: NetworGame: A versatile spatial game program package

Another modeling strategy that reveals key aspects of network dynamics is that of spatial games. In these, agents playing repeated rounds of social dilemma–type games, such as the prisoner's dilemma game, can play only with their neighbors. Spatial games reveal the level of overall cooperation achieved by the complex system, as well as the contribution of individual nodes to this cooperation. Cooperation, in the dynamic and complex setting of spatial games, is a good example of the emergent properties of networks. Emergent properties cannot be predicted from the behavior of individual nodes, but characterize the functions of the complex system encoded by the network structure. Although spatial games are generally played by conscious agents (humans), spatial games can be used for understanding networks of nonconscious agents, such as amino acids, proteins, or signaling components. These game theory–based simulations reveal the degree of cooperation within the complex system (*16*, *25*). Strategies that combine learning and creativity (wherein the former was introduced by taking into account the outcome of several previous rounds of the game in the definition of the strategy update rule of the agent and the latter was modeled by introducing a small amount of random noise to the strategy update rules) have been shown to induce a great amount of cooperation irrespective of network topology (*25*). We have developed NetworGame, a versatile program package that models any type of two-agent game with two to five strategies in any real-world or model network by using any type of strategy update rules, update dynamics, and starting strategies. The NetworGame algorithm is freely available at http;// www.linkgroup.hu/NetworGame.php.

Slide 12: Modular overlaps and bridges are key determinants of network cooperation

To illustrate the power of spatial game analysis and to show the decisive role of bridges in determining network-wide cooperation, we analyzed Michael's strike network (*26*). Nodes in this network are workers in a small forest product factory, and links represent the workers' social contacts. The leadership of the factory wanted to introduce a new set of rules, and convinced the union leaders, Sam and Wende (marked with red dots), on the benefits of this new work scheme. Despite this agreement, however, a strike broke out. The director hired a sociologist, who assembled the sociogram shown in the figure and suggested that the leadership try to convince Bob and Norm, who were workers that occupied central positions within the network. Bob and Norm were designated "BC" for their high betweenness centrality in the network: They connected the three communities of young, old, and Hispanic workers. The strike ended when Bob and Norm had been convinced. By simulating the worker-to-worker cooperation that was needed to start and maintain the strike as the role of cooperating doves in a hawk-dove game, and by selecting Sam and Wende as the only noncooperating (strike-breaking) hawks at the beginning of the game, their small influence resulted in a mostly cooperating (dove) community that together resisted the new work scheme and supported the strike. In contrast, by selecting Bob and Norm as the only noncooperating hawks in the starting strategy distribution, the community became predominantly noncooperating (strike-breaking, hawk), which shows the large influence their bridging position had on the dynamics of the whole system. In addition to the hawk-dove game, other simulations of spatial games, like the prisoner's dilemma or stag-hunt game, have also illustrated the influence of modular overlaps and bridges in the determination of the cooperation of biological networks, such as protein structure networks or interactomes (*27*).

Slide 13: Adaptive rearrangements of the yeast interactome as a model of systems-level signaling

Changes in gene expression patterns after heat shock, an archetype of stress, represent a widely used model for adaptive response (*28*, *29*). We assessed the systems-level rearrangement of a yeast protein-protein interaction network derived from the BioGRID interaction database [http://www.thebiogrid.com (*30*)] that consisted of 5223 nodes and 44,314 interactions after a variety of stress conditions, including heat shock. Link weights of the unstressed yeast interactome were calculated from the abundances of mRNAs encoding the individual proteins (*31*), and stress-induced changes of link weights were approximated by using changes in the abundance of these mRNAs (*32*). Several alternative methods have been used for calculating the initial and stress-induced network link weights. Our most important finding, that the modules of the yeast interactome partially disassemble after stress, was a key discovery that was supported by using link weights calculated by mRNA abundance or any of the alternative methods for calculating link weights (*29*, *33*).

Slide 14: ModuLand: A family of methods for detecting overlapping network modules

The determination of network modules (in other words, network communities, a group of nodes having a greater link density than others in their neighborhood) is a rather enigmatic, key problem of network studies. After initial efforts provided a clear "yes" or "no" assignment of nodes to various modules, recent studies emphasized the importance of overlaps (*34*, *35*), which represent well the multitude of protein functions exemplified by signaling crosstalk (*4*). We analyzed the overlap between the modules of the yeast interactome by using a novel method, the recently published ModuLand framework (*35*). In the figure, we show the four major steps of the method using the network of network scientists as assembled by Mark Newman (*36*). This network represents the collaborative interactions between 379 scientists studying networks as defined by their coauthorships. In the first step of the method, local influence zones of each set of links (or nodes) were defined. The influence zones A1, A2, and A3 show the influence of the collaborative links of Barabási and Vicsek (A1), Girvan and Newman (A2), and a composite subnetwork of the Arenas and Pastor-Satorras collaborative link merged with that of Guimera and Amaral (A3). All these well-known network scientists had a rather large influence zone as determined by the LinkLand method, one of the ModuLand algorithms. Panel B illustrates the community landscape, in which the vertical scale is the sum of the influence zones containing the given link (or node). The value of the vertical scale also represents community centrality, because it characterizes the influence of the entire network on a given link or node. Panel C shows the overlapping modules as colored hills of the community landscape determined by the TotalHill algorithm, one of the module membership assignment methods in the ModuLand collection. Panel D illustrates a coarse-grained representation of the network in which the nodes correspond to the modules of the original network and the link weights denote the extent of overlap between modules (*35*). The algorithms of several versions of the ModuLand program package (including all those mentioned herein) are freely available at the Web site http:// www.linkgroup.hu/modules.php.

Slide 15: Modular overlaps as keys of adaptation processes

Modular analysis of the rearrangements of the yeast interactome showed that the overlap between the modules decreased significantly (Wilcoxon paired test, $P < 2.2 \times 10^{-16}$) after heat shock. Similar decoupling of interactome modules was observed after other types of stress, such as oxidative stress, nutrient limitation, and hypo- or hyperosmotic stress. This reduced overlap reflects a lower density of short- and long-range interactions between large protein assemblies (mega-complexes) of the yeast cell during stress. This stress-induced decrease of intermodular connections is beneficial to the cell, because it allows better focusing on vital functions, thus sparing resources, and localizes damage (by free radicals, for example) to only the most sensitive modules. It also reduces the propagation of noise throughout the network, allows the individual modules a larger degree of freedom for exploring different adaptation strategies, and helps reduce intermodular conflicts during a period of major intramodular changes. Therefore, modular overlaps emerge as keys to adaptive processes in cells, as well as in other complex systems, including social networks (*28*, *29*, *33*).

Slide 16: SignaLink: A high-quality signaling network database

To assess the complexity of signaling in three organisms—*Caenorhabditis elegans*, *Drosophila melanogaster*, and humans—we assembled a manually curated signaling network database called SignaLink. The network contains 1550 components and cofactors of the following eight major signaling pathways: epidermal growth factor–activated mitogen-activated protein kinase (EGF-MAPK), nuclear hormone receptor (NHR), Janus kinase–signal transducer and activator of transcription (JAK-STAT), Hedgehog (HH), Notch, insulin and insulin-like growth factor (IGF), transforming growth factor–β (TGF-β), and Wnt (*4*). The database is freely available at http://www.SignaLink.org. The April 2011 version of the database predicted 253 previously unknown signaling orthologs ("signalogs") in the three organisms (*37*). (Signalogs are proteins that are predicted to participate in a signaling pathway in one organism on the basis of their homology to similar proteins that participate in the same signaling pathway in another organism.) Signalogs can be searched and viewed by using the online tool available at http://www.signalink.org/signalog. Each of the six predicted, previously unknown signalogs of the *C. elegans* Notch pathway have been experimentally verified to participate in *C. elegans* Notch–dependent vulval development in the expected manner (*37*).

Slide 17: Overlaps of signaling pathways are most pronounced in humans

In this figure, crosstalk between the eight pathways in the SignaLink database (*4*, *37*) are represented by links in which the width of a link is proportional to the weighted number of directed signaling interactions between the two pathways. The figure shows that crosstalk was greater in *D. melanogaster* (panel B) than in *C. elegans* (panel A). Most importantly, in humans (panel C), any two pathways were found to interact with one other. Crosstalk between classes of signaling components from different pathways (such as ligands, receptors, mediators, cofactors, and transcription factors) showed an even more dramatic increase—mostly between differing classes of signaling components—in *D. melanogaster* (panel E) as compared with *C. elegans* (panel D). Here again, all classes of signaling components of pathways were found to interact with all classes of signaling components of all the other seven pathways in humans (panel F), whereas crosstalk in the other two species was mostly limited to cofactors (proteins that modulate the function of other signaling proteins) and mediators [pathway members that mediate signals from receptors to transcription factors (*4*, *37*)]. These observations illustrate the importance of modular overlaps and bridges in human signaling pathways.

Slide 18: Summary: Modular overlaps and bridges as potential drug targets

In summary, our studies showed that modular overlaps (nodes or links belonging to two or more modules) and bridges (nodes or links having a significant portion of their neighborhood in two or more modules) are the primary transmitters of cellular perturbations and signals and are key determinants of cooperation between network nodes and modules. Overlaps and bridges are also the predominant sites of modulation during cellular adaptation, such as during stress, and are prevalent in human signaling processes. These properties of modular overlaps and bridges all suggest the importance of these proteins as potential drug targets.

Slide 19: Multipathway signaling proteins as established and potential drug targets

We assessed the prevalence of multipathway proteins of the eight major human signaling pathways represented in the SignaLink database (*4*, *37*): the modular overlaps and bridges of the human signaling network that would represent good potential drug targets. For this assessment, the following four common determinants of drug target molecules were taken into account: membrane localization, enzyme activity, presence of kinase domains, and association with diseases. The figure shows that 13 of these human multipathway proteins have three or four of these drug target–like properties. Six of these are, in fact, established drug targets: EGFR (epidermal growth factor receptor), IGFR1 (insulin-like growth factor 1), and the kinases AKT1, GSK3B, MEK1, and p38a (*4*, *37*).

Slide 20: Modular overlaps imply the necessity of multitarget drugs

Modules of protein-protein interaction and signaling networks often correspond to cellular functions, such as protein synthesis, protein degradation, and signaling responses. A large number of cellular proteins belong to multiple modules of either protein-protein interaction or signaling networks, or both. Thus, the complete inhibition of a protein may decrease the efficiency of many cellular functions at the same time. In contrast, efficient inhibition of a particular cellular function can often be accomplished only by the inhibition of many proteins, wherein the inhibition of each protein is only partial (*20*, *34*, *35*). Multitarget drugs often have a parallel inhibition of alternative pathways, thus requiring a smaller dose for efficacy and therefore exhibiting lower toxicity (*20*, *38*). The partial inhibition of proteins converts their high probability, high affinity, strong links to lower probability, lower affinity, weaker links. Because of the general stabilizing role of weak links in complex networks (*9*, *10*), multitarget drugs may stabilize the biology of otherwise perturbed cells in the diseased organism (this effect is summarized as "more weak links: more stable cell" on the slide). This is in contrast to the action of many single-target drugs, which often enhance the destabilization of affected cells and by this action may hinder the recovery of the individual from disease (*20*, *38*).

Slide 21: Multitarget drugs are target multipliers

Single-target drugs usually target those proteins that are predominantly associated with a disease. This limits the pool of potential targets for future drug development. Moreover, many of these disease-associated proteins are not druggable in the sense that they do not have a hydrophobic binding site for a potential orally delivered, membrane-permeable drug molecule. This restricts the number of available drug targets even further. In contrast, the application of multitarget drugs increases the likelihood of effec-

tive treatment by targeting multiple proteins, each of which may only slightly influence the disease-associated proteins through signaling crosstalk, for example. Moreover, the eased constraints of low-affinity binding allow the targeting of partially hydrophilic binding sites by orally deliverable, hydrophobic molecules. This dual increase of both the pool of disease-associated proteins and the pool of proteins binding orally deliverable, hydrophobic molecules predicts a very promising increase of the number of proteins in the overlap of the two pools (the proteins that are both disease-associated and available for targeting by orally delivered drugs) and allows the exploration of novel drug target families. Thus, multitarget drugs are, in fact, target multipliers (*20*, *38*).

Slide 22: Network analysis as an option to identify alternative multitarget sets

The development of multitarget drugs is a very promising area of drug design. However, the experimental identification of a set of target proteins is a much harder task than the already difficult identification and targeting of a single disease-related protein. This is why systems-based approaches, and, in particular, network analysis, may be extremely helpful to find target sets for developing multitarget drugs. The application of the tools for analyzing network topology and dynamics introduced in this study may help to identify alternative target sets, the inhibition of which by a multitarget drug selectively inhibits the set of proteins primarily associated with the disease (*21*).

Slide 23: Acknowledgments

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