Fault Diagnosis Engineering in Molecular Signaling Networks: An Overview and Applications in Target Discovery

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Fault Diagnosis engineering is a key component of modern industrial facilities and complex systems, and has gone through considerable developments in the past few decades. In this paper, the principles and concepts of molecular fault diagnosis engineering are reviewed. In this area, molecular intracellular networks are considered as complex systems that may fail to function, due to the presence of some faulty molecules. Dysfunction of the system due to the presence of a single or multiple molecules can ultimately lead to the transition from the normal state to the disease state. It is the goal of molecular fault diagnosis engineering to identify the critical components of molecular networks, i.e., those whose dysfunction can interrupt the function of the entire network. The results of the fault analysis of several signaling networks are discussed, and possible connections of the findings with some complex human diseases are examined. Implications of molecular fault diagnosis engineering for target discovery and drug development are outlined as well.

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1. Introduction. -- Enormous amount of information regarding the function of individual signaling molecules have been collected over the past few decades. We now have access to the many detailed individual molecular mechanisms regulating different cellular functions. Despite the remarkable advances in our knowledge of cellular and molecular biology and biochemical interactions driving many cellular functions, effective therapeutics for some of the most common human diseases are still not known. Examples include cancer and some complex psychiatric and neurodegenerative human brain disorders manifested by cognitive impairments such as schizophrenia, autism, affective disorders, Alzheimer disease, different types of dementias, etc. Due to the complexity of these disorders, it is now widely accepted that the pathology arises from the failure of a

highly sophisticated biological system and not from a single molecule or a specific pathway. Therefore, in order to move towards the treatment in the right direction, systems biology approaches should be developed, in which all the molecules and pathways are considered together. In such approaches, the disease can be considered as the outcome of a failed or faulty complex system [1], composed of numerous interconnected components, where some of them are dysfunctional. To understand why and how the system has failed, one needs to find out how and to what extent each individual molecular element contributes to the breakdown of the whole system. This allows to dissect out how numerous different components of a variety of signaling pathways contribute to the development of a disease, when they function together in a fully-connected network. It is crucial to figure out how important the role of each component is, to the ultimate failure of the whole system. This in turn will tell us how much emphasis we should put on those molecules which are identified as major contributors, in the stage of drug development. Identification of disease-related pathways and molecules using a systems biology approach has the potential to help minimizing the side effects of drugs as well [2].

Before reviewing the basic concepts of fault diagnosis engineering, it needs to be pointed out why molecular "*signaling*" networks are important to analyze. There is already a large body of knowledge showing the involvement of many signaling molecules in different human diseases [3]. The main advantage of considering signaling molecules is that they are "*regulatory*" molecules, mostly enzymes, that are involved in regulating almost all aspects of the cellular function. Generally, they are ubiquitously expressed in different tissues and highly conserved between different species. They are the major regulators of the function of other proteins as well. That is why they have been emerged as targets for developing new therapeutics [4].

2. An Introduction to Fault Diagnosis Engineering. -- Accurate fault diagnosis of complex computer chips and processors, microelectronics manufacturing processes, pharmaceutical/drug manufacturing facilities, production lines, control systems, power

plants, etc., is of high importance, in order to minimize the costs and prevent the risks of system failure [5] [6] [7] [8] [9] [10]. Fault Diagnosis and monitoring, also known as risk assessment and reliability analysis, are key parts of modern industrial facilities and complex systems, and have gone through considerable development in the past few decades. Now they are inevitable parts of modern industrial processes and man-made complex systems. For example, early and precise fault diagnosis in a nuclear power plant is critical for the plant to safely operate, and to avoid significant financial loss. Other examples include microelectronics and chemical manufacturing industries, as well as highly complex systems such as space shuttles.

A fault is defined as an abnormal change of one or several system nodes and variables from their normal operating conditions and states. Due to the large number of interconnected nodes in a complex system, it is not a trivial task to correctly localize a fault. Mathematical theory of systems has been the starting point of fault diagnosis engineering. For small systems with well-characterized system equations and models (input-output or state-space differential equations), it is possible to use the system model for fault diagnosis [8]. In such methods, a mathematical description of the system together with the inputs and the resulting outputs are used to generate information about the system. This information, say, the residuals generated based on the system model, can be used for fault detection and isolation [9]. However, even in systems for which precise models and equations are known and available, computational complexity of a fault diagnosis algorithm could be prohibitive. For example, thermal modeling and monitoring of low pressure chemical vapor deposition furnace is of interest in semiconductor manufacturing, to have a tight control on film uniformity [11]. However, fault diagnosis in such a system where its model includes hundreds of partial and ordinary differential equations can take a very long time and computational complexity is enormous.

The situation becomes much more difficult in very complex systems where there are so many complex interactions and system variables, with many parameters involved.

For such complex systems, methods that work for small systems are not practical. One promising way is to use artificial intelligence and expert system type techniques [7]. Such methods rely on less-detailed system models such as logical and Boolean models [12], in order to make the fault analysis feasible.

3. System Characterization of Signal Transduction Networks for Fault Diagnosis. -- There are different types of models for intracellular signaling and signal transduction networks such as Boolean models, differential equations, graphs, etc. [13] [14]. Each of these models represents some aspects of signal transduction networks, and it is the research question of interest that specifies what model and representation should be used. For a fault diagnosis type study, to determine how the failure of each molecule affects the function of the entire molecular network, one needs to first define what it means for a molecule to be faulty. The failure of a molecule to function correctly appears in different forms. For example, if there are changes in the shape or the conformation of a specific molecule due to the mutation in the gene encoding that particular molecule, this misfolding will affect the optimal activity of that protein within the network. As another example, if the kinetics or binding constants of a protein change, they will also be reflected in the activity of that particular protein. Or if the concentration of a particular molecule is low, the overall activity of that molecule in the network will be lower accordingly. So, one approach to fault analysis is to think of all possible biological/physical/chemical abnormalities and defects, which entails many different types of faults. However, this makes the search space for fault analysis very large and perhaps unmanageable, specially in large networks with hundreds of molecules. To avoid this problem, it is worthwhile to have a small number of biologically relevant faults.

If we think about the outcome of different types of abnormalities in molecules, we can summarize the end results of different forms of molecular failures in the system as unwanted changes in the "activity" of molecules. So, a signal transduction network model which is built upon the activity of molecules is suitable for fault analysis, since it

provides a manageable size for the fault space of the network (as explained later). From this perspective, Boolean modeling serves as an appropriate choice for fault analysis, since it models the state of each molecule as being either "active" or "inactive," controlled by the regulatory inputs of the molecule (Fig. 1a). In Boolean logic terms, each molecule is modeled to be 0 (inactive, off) or 1 (active, on). A molecule can then be defined to be faulty or dysfunctional, if its activity is stuck at a certain state and does not change in response to its inputs (Fig. 1b). This gives rise to two types of faults [15]: stuck-at-0 and stuck-at-1. This small number of faults provides a manageable size for the fault space of the network. Therefore, one can find critical molecules, those whose dysfunction severely affect the function of the network, by fault analysis of the Boolean model [15]. Once critical molecules are identified, one can go back and check the physical basis for the possible faulty behavior of each critical molecule. For example, the stuck-at-0 fault for a protein might be due to a mutation that results in the misfolding of that protein, or could be because the protein is not expressed. In this example, both abnormalities are modeled by a single stuck-at-0 fault model (a macroscopic view), which makes the fault analysis feasible. After the fault analysis, one can focus on each critical molecule to understand what may physically go wrong which makes that molecule dysfunctional (a microscopic view).

Boolean models have been extensively studied, both computationally and experimentally, to explore different characteristics of signaling and genetic networks [15] [16] [17] [18] [19] [20] [21]. They also have a certain prediction power which is validated by experiments. Here prediction power means the ability to correctly specify the network output using the model, in response to inputs. Boolean modeling has its own applications and similar to other models, it cannot be used to model all characteristics of signaling networks. In general, the usefulness of any modeling approach depends on the goal of the study. As explained above, Boolean models offer a picture of cell signaling which is

sufficient for fault analysis and diagnosis, and provides results that are consistent with experimental data [15] [18] [22].

4. Fault Diagnosis and Reliability Analysis in Electronic Circuits. – Since the early days of electronics, faulty parts such as diodes and transistors were known to contribute considerably to the failure of electronic devices and systems [23]. In the world of computers, very large scale integrated (VLSI) electronic circuits were introduced in 1980's with hundreds of thousands of transistors [24]. Nowadays VLSI chips with millions of transistors are used in almost every complex electronic device, ranging from computers to cell phones to digital TVs to state of the art medical equipments, etc. Having many millions of transistors and wires with nano-scale dimensions on a single chip makes the chip vulnerable to manufacturing defects.

In the nano-world of a chip, a very small manufacturing flaw can result in a disconnected wire or defective transistor, which can make the chip dysfunctional. In a manufacturing facility, a chip is manufactured based on a particular design and is supposed to provide a specific function. However, during the fabrication process, physical defects such as faulty transistors, open and short wires, etc. may happen, which cause the manufactured circuit not to function correctly [5] [6]. Testing of chips and digital circuits is therefore necessary to separate defective manufactured parts from the non-defective ones, to guarantee the delivery of fault-free products to the end user. The test itself is an assessment of the manufactured circuit, according to a set of criteria. During the lifetime operation of electronic systems, the correct operation is a key aspect and is typically referred to as reliability.

5. Fault Diagnosis and Vulnerability Assessment in Molecular Networks. – It is well recognized that there are many similarities between electronic circuits and signaling networks [25]. This, together with the lessons learned from the mature field of circuit fault diagnosis discussed previously, has provided sufficient motivation to develop a fault diagnosis engineering framework for intracellular signaling networks [15]. In this

framework, roughly speaking, each cell resembles a chip, molecules are like transistors and biochemical reactions represent wires. Inspired by digital circuit fault analysis and reliability engineering methods, one can find the critical molecules of complex signal transduction networks. Critical, or equivalently, highly vulnerable molecules are those whose dysfunction seriously affect the function of the entire network. The ultimate goal is to find the most promising molecular targets for the treatment of some complex human disorders where the molecules which may have causative effects are not known. These disorders include a variety of psychiatric and neurodegenerative brain disorders such as autism, schizophrenia, affective disorders, Alzheimer disease and different types of dementias, as well as different types of cancer, metabolic disorders such as diabetes, obesity and cardiovascular diseases, etc. The computational results and experimental support provided by Abdi *et al.* [15] have demonstrated the utility of fault analysis approach in finding critical molecules of intracellular signaling networks, i.e., molecules whose dysfunction are detrimental to the overall function of the network.

To determine the most vulnerable molecules in a network of molecules, in [15] a class of electronic circuit reliability analysis techniques known as vulnerability assessment methods is used. Such methods compute numerical values for the vulnerability of the function of the entire molecular system to the dysfunction of each individual molecule. A high vulnerability for a molecule means that with high probability, the whole signaling network does not function correctly, if that molecule is dysfunctional. Identification of such important molecules in signaling networks implicated in disease is a key step towards understanding the molecular basis of complex human diseases. From the drug development point of view, vulnerability assessment provides a set of candidate molecules to target.

To implement the algorithm, one needs to first specify the output nodes of the network. Output nodes could be some specific transcription factors or some cellular functions and processes that are relevant to the disease of interest. For instance, in cancer related studies,

one may want to investigate a network whose outputs are widely accepted important regulators of cell proliferation and cell cycle. The tumor suppressor p53, a transcription factor for several genes that control apoptosis [26], can therefore be considered as the output node [15] for such studies. Or, one may want to analyze a network that regulates mitosis [18]. As another example, to study human disorders manifested by memory dysfunction such as Alzheimer disease [27] or schizophrenia [28], one can choose the network output to be the transcription factor CREB (cAMP responsive element binding protein) [15]. This is because of the physiological role of CREB in neuronal mechanisms underlying the memory function of mammalian brain [29]. Input nodes of the network can be ligands, receptors or secondary messengers that are relevant to the output nodes. For example, the input nodes of the CREB network [15] are chosen to be seven major ligands in nervous system, i.e., glutamate, dopamine, γ-aminobutyric acid (GABA), serotonin, acetylcholine (ACh), adenosine, and enkephalin.

Once input and outputs nodes of the network are specified, all relevant intermediate molecules that allow the signals to propagate from the inputs to the outputs should be added to the network. Furthermore, stimulatory or inhibitory interactions among the molecules need to be included in the network as well. The next step would be the conversion of the reconstructed network to an equivalent electronic circuit, which can be further analyzed, to find the most critical nodes (molecules). All these steps are discussed in Abdi *et al.* [15] in detail and several examples of small and large networks are analyzed there.

The above molecular vulnerability assessment algorithm computes the molecular system vulnerability to the dysfunction of each molecule within the system. Here we provide a summary of the findings of this fault analysis approach and their biological relevance. In Fig. 2 four molecular networks are shown. The first one controls the activity of the transcription factor CREB, the next two regulate the transcription factor p53 and the mitosis process, respectively, and the last one controls caspase3 and forkhead-related transcription factor (FKHR). In what follows, each network and its critical molecules,

obtained using the vulnerability assessment algorithm are briefly discussed. Keep in mind that by definition, the vulnerability value of a molecule is the probability that the system fails (incorrect system output), if that particular molecule is dysfunctional or faulty.

The CREB network (Fig. 2a): The output node of this network is the transcription factor CREB (cAMP responsive element binding protein) and the input nodes are seven major ligands in nervous system: glutamate, dopamine, GABA, serotonin, ACh, adenosine, and enkephalin. This network is comprised of 64 molecules and 152 intermolecular interactions [15]. Vulnerability assessment has shown that dysfunction of 41 molecules out of 64 will not contribute to the failure of CREB network (vulnerability values less than 0.1). The molecules with a vulnerability of more than 0.5, which indicates that their dysfunction can result in the failure of CREB function, are calmodulin, calcium, cAMP, Gαi, AC1, AC2, AC5, PKA, P/Q-type calcium channel, and PP2A. These molecules can be grouped into elements of the cAMP-dependent signaling (cAMP, Gαi, PKA, and the AC isoforms) and elements of calcium signaling (calcium, calmodulin, and P/Q-type calcium channels). These molecules are functionally related molecules, and some of them are already known as main physiological regulators of CREB function. Indeed the name CREB comes from the identification of the protein as a cAMP responsive element binding protein. This engineering analysis has identified cAMP and the molecules directly related to cAMP function, such as AC1, AC2, AC5, and PKA, as the most critical molecules for the regulation of CREB. The crucial role of PKA in the regulation of CREB is well known [29]. Important functions of the cognitive and executive human brain, such as learning and memory, are directly regulated by cAMP-dependent CREB functions [29]. In pathological terms, direct evidence for deregulation of PKA signaling has been reported in human disorders manifested by memory dysfunction, such as Alzheimer disease [27] or schizophrenia [28]. Vulnerability assessment of the CREB network has also identified some elements of calcium signaling as playing a major role in the function of CREB. This observation is also physiologically

and pathologically relevant and consistent with experimental data [30]. Furthermore, several pathological conditions associated with memory dysfunction can arise from deregulation of calcium-dependent signaling [31] [32]. It is not yet clear how CREB is involved in the pathogenesis of these disorders, the physiological role of CREB in neuronal mechanisms underlying the memory function of mammalian brain has been known for many years [29].

Many of the above highly vulnerable molecules identified using the fault analysis approach are already experimentally known regulator of CREB. This validates the ability of the approach in finding critical molecules. Identification of Gαi and the P/Q-type calcium channel as critical regulators of CREB, however, are new findings which are experimentally validated in Abdi *et al.* [15]. The ability of the constructed Boolean network model to correctly predict the output activity in experimentally verified in [15], as well as the biological relevance of the stuck-at-1 and stuck-at-0 fault models.

The p53 network (Fig. 2b): This network has a total of 49 molecules, with 94 intermolecular interactions. The input nodes are the two ligands insulin and PDGF, and the output node is the transcription factor p53. The p53 network shows the highest vulnerability (more than 0.5) to the dysfunction of PIP2, AKT, caspase3, and PP2A [15]. Previous studies have experimentally shown that these molecules are known to be the key regulators of p53 (see the references listed in Abdi *et al.* [15]). Similar to the CREB network, a majority of the nodes (38 out of the 49) have exhibited very low vulnerability values (less than 0.1).

p53 has been found mutated or functionally inactivated in more than half of all the human cancers [26]. The four molecules that have shown the highest vulnerability values in the p53 network are known to be key regulators of the cellular functions for which p53 is responsible. For AKT and caspase3, there is clear evidence that these two molecules play crucial roles in regulating a number of p53-regulated functions, such as cell survival and apoptosis [26] [33]. More importantly, there is experimental evidence supporting a

key role for AKT in proper activation of p53 [34]. Although PIP2 have not been directly connected to p53, it is a regulator of AKT and thus this connection to AKT may explain its high vulnerability value. The experimental evidence that PP2A and caspase3 plays a major role in regulating p53 activity comes from [35] [36]. Thus, molecules diagnosed as highly vulnerable molecules by this approach are experimentally known to contribute to the failure of the p53 network and cause the pathology in humans. This demonstrates the reliability and biological relevance of the approach.

The mitosis network (Fig. 2c): This is a signaling network in capillary endothelial cells that regulates cell cycle and is experimentally verified by Huang and Ingber [37]. It includes a number of functional and molecular nodes. The output node of this network represents mitosis, a process involved in cell division, whereas input nodes are GF (growth factors) and spread, which represents the cell shape (spreading). The total number of interacting molecules and processes in the mitosis network is 11, with 18 interactions among them. Vulnerability analysis of this network has shown that p27 has the highest vulnerability [18]. This observation is biologically and pathologically relevant since the critical role of p27 in regulation of cell cycle has been reported and it is now widely accepted that reduced p27 levels directly correlate with tumor aggressiveness and poor patient survival [38].

The caspase3-FKHR network (Fig. 2d): This is a network which is experimentally verified by Janes *et al.* [39]. In this network there are three input ligands EGF, insulin, and TNF, two outputs caspase3 and FKHR, and 17 intermediate nodes with 27 interactions among them. According to the vulnerability assessment results, this network shows the highest vulnerability to the dysfunction of AKT [15].

6. Graph Theory and Sensitivity Analysis. -- When it comes to finding important molecules, one may think of graph theory and sensitivity analysis. In graph theoretical approaches, a molecular network is represented by a graph of connected nodes, where each node represents a molecule. Typically the topology of the graph and connections

among the nodes are the basis for analysis and making predictions [40] [41], without including the functional/biological relationships between the molecules. For example, pathways with the shortest path length from input to output may be considered as important pathways. An another example, hubs (nodes with many links and connections) are sometimes considered as the most important nodes, although there are studies which suggest a low correlation between the importance and number of connections of a node [42]. Different types of centrality parameters such as degree centrality, closeness centrality, betweenness centrality and feedback-based centralities [40] [41] can be used for graph theoretical studies.

In sensitivity analysis studies, usually the sensitivity of the molecule concentration levels to the variations of rate constants and kinetic parameters are calculated [43] [44]. Molecular networks in such studies are typically modeled using differential equations. In large signaling networks, there are many interactions, as well as many parameters and system variables. To conduct a sensitivity analysis, the normal values of these system variables and kinetic parameters should be known. In the area of fault diagnosis engineering, one way to handle this issue is to use less-detailed system models for the fault diagnosis of complex systems. In other words, although all the system equations and nominal values of the parameters are known, sometimes it is preferred to use less complex system representations such as Boolean. These simpler models capture essential features of the system under normal operating conditions and make fault diagnosis more feasible, when the system is dysfunctional.

7. Target Discovery in the Light of Fault Diagnosis Engineering. -- Successful research and development for a novel therapeutic typically cost about one billion dollars. This includes ~$250 M for a drug that successfully enters the market, plus ~$750 M for those that fail at different stages of development and trials. This means that the vast majority of the enormous cost of R&D for new drugs comes from the failure of a candidate drug, mostly because of its toxicity [45]. This fact underscores the necessity of

developing novel technologies that can identify molecular targets with minimum chance of failure during the long term process of clinical trials.

Targeting the activity of different enzymes has been a strategy for the development of novel therapeutics for many years [4]. However, curative treatments for some of the most common human diseases such as cancer, neurodegenerative and psychiatric brain disorders are still unknown. This is because the pathology of such complex disorders results from the failure of a highly sophisticated biological system and not just a single molecule or a specific pathway. So, to understand the pathology and move towards effective treatments, a systems biology approach needs be developed, to study the orchestrated function of complex interconnected molecular pathways.

The recently developed fault diagnosis systems biology approach allows to find novel critical regulators in complex cellular networks and can accurately predict which molecules are "faulty" when the system fails to function correctly [15]. This system engineering approach is capable of predicting which molecules in large cellular networks are the most critical ones for the development of disease conditions. Moreover, as described in [46], this fault diagnosis approach can identify the most promising therapeutic targets for the treatment of some complex trait disorders.

On the other hand, various computational algorithms have been developed to explore transcriptional networks and modules from gene expression data. *In silico* studies have also been made to model the dynamic behavior of regulatory networks and eventually identify how disease or cellular phenotypes arise from the connectivity or networks of genes and their products. A recent review article [48] nicely reviews the recent development in computational biology research on such dynamic behaviors of transcriptional networks, and also demonstrates how these computational algorithms can be applied to systems biology studies on disease, stem cells, and drug discovery [47] [48]. Furthermore, some basic computational studies based on protein-protein interactions [49] have presented specific methods for the integration of various data sets to predict

transcriptional regulatory interactions and describe how to accumulate various data sets from different sources and how to predict such transcriptional regulatory interactions by homology-based approaches. Such methods use integrated data sets that include experimentally verified transcriptional regulatory interactions, binding sites of transcription factors, promoter sequences, protein subcellular localization, and protein families. These approaches give insight into specific pathways, network motifs, and the topological dynamics of an integrated network with gene expression under different conditions, which potentially can pave the way for the development of better targeting strategies [49].

Several other computational methods have been developed and widely applied to pharmacology, drug development and testing. Examples of the computational methods used here are development of databases, quantitative structure-activity relationships, similarity searching, pharmacophores, homology models and other molecular modeling, machine learning, network analysis and data mining tools that use a computer. Such methods have seen frequent use in the discovery and optimization of novel molecules with affinity to specific targets [50].

More advanced computational methods have also been developed to predict the central nervous system (CNS) penetration of chemical structures. In neuro-pharmaceutical settings, it is very important to determine whether a compound will penetrate and distribute within CNS, with the required pharmacokinetic and pharmacodynamic performance. *In silico* predictive methods are used for such settings and are affected by the quality, quantity, sources, and generation of the measured data available for model development [51].

Computational approaches have been used to understand the pathogenesis of both infectious and genetic diseases in humans. For example, complete genome sequences are now available for three members of the Mycobacterium tuberculosis complex and the related intracellular pathogen M. leprae. Several predictions generated by computational

analysis on the genome of such organisms have been validated through functional analysis and have led to the identification of essential genes involved in the development of the disease. Such studies can define potential targets for new and existing drugs and also can help in tuberculosis vaccine development, by pinpointing potentially antigenic proteins, as well as providing better diagnostic tools to detect infection [52].

Overall, research and development for new drugs and clinical trials are now extremely expensive. Hence, development of novel methods that can minimize the time and also the chance of failure are greatly needed. The fault diagnosis systems biology approach is capable of identifying effective and safe molecular targets for treatment of some complex human disorders. Some preclinical studies are needed to further validate the capability of the approach in predicting safe molecular targets. Such systemic approaches prevent spending so much money and time on targets that can turn out to be toxic after several years of work. That is why the fault diagnosis approach can minimize the cost of R&D for new medications. It can be applied to molecular networks involved in a variety of complex human disorders, such as mental disorders, cancer, diabetes, autoimmune diseases, etc., to find safe molecular targets for treatment of such disorders.

8. Conclusions. – Systems biology approaches developed based upon electronic circuit fault analysis methods allow biologists to determine the vulnerability of intracellular signal transduction networks to the dysfunction of molecules within the network. For proper propagation of the input signals to regulate the activity of the network outputs, normal function of highly vulnerable molecules is necessary. When highly vulnerable molecules are faulty, the network does not correctly convey the input signals to the outputs. So, the molecular network does not function properly. Experimental data indicate that fault diagnosis engineering can correctly predict new critical components of molecular networks, and also can correctly identify previously known critical molecules. Vulnerability analysis results of four signaling networks are briefly presented and involvement of identified highly vulnerable molecules in some

complex human disorders is discussed. Implications of fault diagnosis engineering for target discovery and development of novel therapeutics are highlighted as well.

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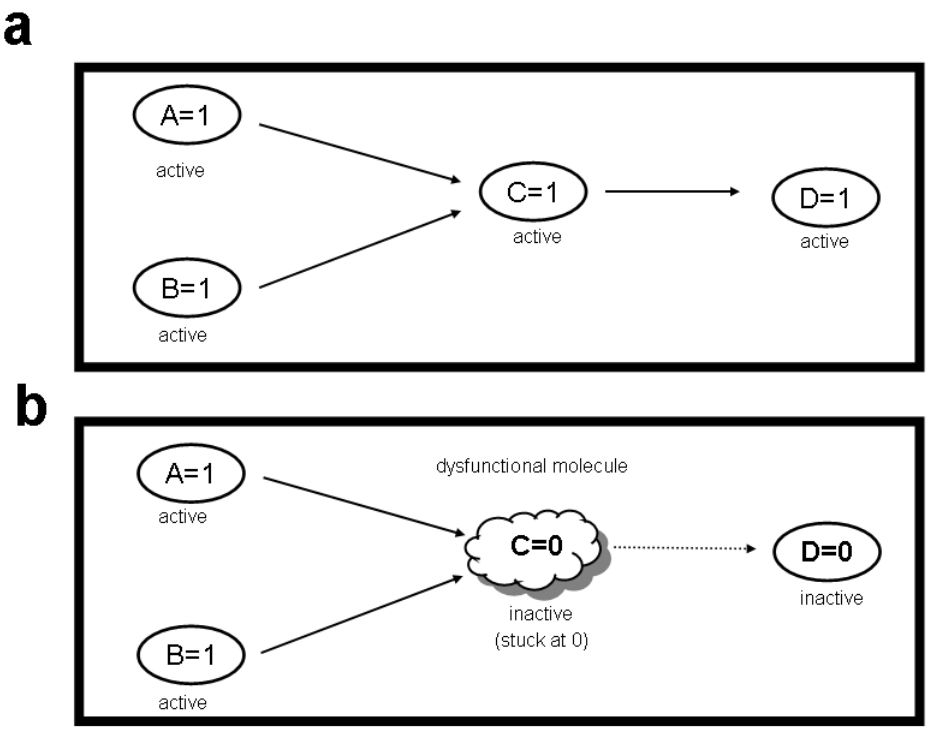

Fig. 1. *A toy network to illustrate Boolean modeling and stuck-at fault concepts:* (a) Signals initiated from the input molecules A and B activate the output molecule D through the intermediate molecule C which functions normally, (b) Signals initiated from the input molecules A and B fail to activate the output molecule D, due to the dysfunction of the intermediate faulty molecule C.

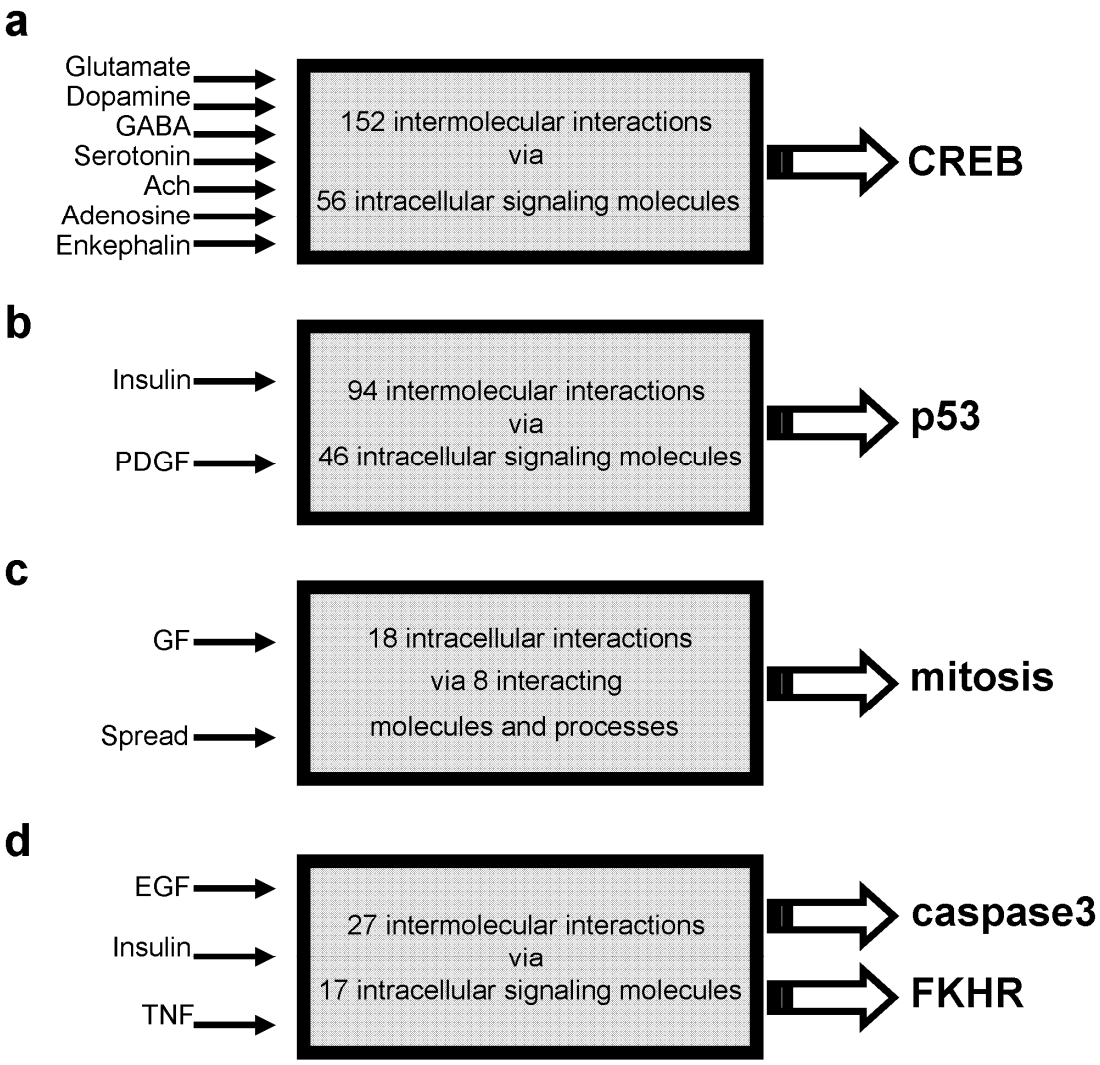

Fig. 2. *Four molecular signal transduction networks analyzed using a fault diagnosis systems biology method [15] [18]*: (a) CREB network, (b) p53 network, (c) mitosis network, (d) caspase3-FKHR network. Inputs of these networks are ligands and other factors that control the activity of output molecules.