Systems Biology: Discovering the Dynamic Behavior of Biochemical Networks

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Abstract : Systems theory and cell biology have enjoyed a long relationship, which, in the context of systems biology, has received renewed interest in recent years. Systems biology is concerned with the dynamic behavior of biochemical reaction networks within cells and in cell populations. The biologist's conceptual frameworks, in which to identify the variables of a biochemical reaction network and to describe their relationships, are pathway maps. A principal goal of systems biology is therefore to turn these static maps into dynamical models. This paper introduces systems biology with the aim of unraveling the dynamic behavior of a biochemical network from different perspectives. Focusing on systems theoretic investigations into biochemical networks, emerging challenges and perspectives in systems biology are discussed.

Keywords : systems biology, biochemical networks, dynamic behavior, signal transduction pathways, mathematical modeling, systems theory, post-genomic challenges

1. Introduction

During the last three years the field of Systems Biology has received considerable international attention of the research community [1, 2, 3, 4, 5, 6]. While in the beginning it was largely associated with some outstanding individuals, working in isolation, it is now accepted as a new discipline open to researchers from a range of disciplines. Numerous centers and professorial positions have been established at Universities worldwide. Together with the appearance of a new focused IEE Journal Systems Biology (www.iee.org/sb) there is sufficient evidence for a longer lasting affair of biology with systems theory.

For any emerging area of research there is a risk that at some point in future it is looked at as a buzzword with all its negative connotations. There are two main causes for this to happen: individuals (mis)use the new term as a means to attract research funding through relabeling old ideas and without actually embracing new approaches. Secondly an area can simply fail, for scientific reasons to realize the promises it made. What is therefore called for is a definition of systems biology that provides a realistic attitude towards the opportunities and hurdles of this field. In our view, systems biology is about methodologies, i.e., data-based mathematical modeling and simulation, that help an understanding of the dynamic interactions of cells and components within cells. For this vision to succeed, we require foremost experiments and technologies that generate quantitative, time-resolved data.

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The area of systems biology, i.e., mathematical modeling and analysis of inter- and intra-cellular dynamic processes using signal- and systems-oriented approaches is gaining momentum with many recent national and international initiatives. The agenda in most research programs in systems biology is to work towards the following questions addressing intra- and inter-cellular dynamics:

- How do the components within a cell interact, so as to bring about its physical structure and biological function?
- How do cells interact to develop higher levels of organization, including tissue and organs?

Advances in our understanding of cancer, cell signaling or cell differentiation, to name only three interesting problems, will crucially depend on our ability to generalize from *in vitro* to *in vivo* situations, from one pathway to interaction of pathways, from one cell to many cells interacting. Mathematical modeling is a prime example of generalization through abstractions. We describe a pathway in terms of mathematical equations. The structure of these equations and the values of parameters establish a relationship to a particular experimental system, say a particular signal transduction pathway. The value of mathematical modeling is that a formal study of these models or their computational simulation allows us to investigate generic properties and "if-then" scenarios. With such methodologies and tools at hand, we can investigate, for example, the role of feedback loops and transport delays that result from the nucleo-cytoplasmic export of molecules. Mathematical modeling is therefore a tool to generate and test hypotheses, thereby supporting the design of experiments.

A principal challenge for the life sciences is to understand the 'organization' and 'dynamics' of those components that make up a living system, i.e., to investigate the spatio-temporal relationships between (macro-)molecules, cells, and tissues, that give rise to cause and effect in living systems. A major problem is that networks of cellular processes are regulated through complex interactions among a large number of genes, proteins, and other molecules. From these considerations, the fundamental goal of systems biology is to understand the nature of this regulation in order to gain greater insight into the mechanism that determine the functions of cells, and ultimately their consequences at a physiological or phenotypic level. In systems biology, this is achieved not through cataloguing and characterizing physical components, but through the integration of this information in mathematical models. Hence, the emergence of systems biology signals a shift of focus away from molecular characterization of the components in the cell to an understanding of functional activity through the interactions in molecular dynamics.

The dynamic relationship between components, their organization, and regulation in complex cellular networks is still largely an open question. The fact that the cell is a dynamic system (or a large set of interacting dynamic processes) requires us in many cases to rethink the way in which we conduct experiments. The apparent success of mining genomic data, has led to large collections of experiments, in which we are looking for differences or 'patterns'. System theoreticians would argue that the 'mining approach' can, at most, identify associations between elements or variables, rendering inferences about causal entailment a scientific art-form that is based on the experience and creativity of the scientist. If it is accepted that system dynamics give rise to biological function, a different approach is necessary to identify causal entailment directly from experimental data. The systems approach is however more demanding on the experimental design in that only a systematic manipulation (through defined perturbations) of the system dynamics will allow us to identify these from experimental data directly. This also implies the need for quantitative, reliable and sufficiently rich data sets.

This paper is devoted to provide a brief survey of some of the methodologies used in systems biology. The paper is organized as follows. In Section 2, definitions of systems biology are reviewed and the systems biology approach is described. Section 3 introduces a general framework of biochemical networks and the complications that are involved. Section 4 reviews dynamic systems approaches to identify the dynamic behavior of a biochemical network based on stimulus-response experiments. Section 5 reviews artificial intelligence approaches. Section 6 reviews other attempts from statistical physics, applying network theory. Finally, challenges and perspectives of systems biology are discussed in Section 7.

2. Systems Biology and the Systems Biology Approach

The most important recent development in the life sciences is that many biological problems are no longer just experimental but are increasingly conceptual. This is largely due to novel technologies that allow us to study many variables simultaneously, from different perspectives and with increasing accuracy. As a consequence, the analysis of data, generated by post-genomic technologies and mathematical modeling, based on these data, is becoming increasingly important [5, 7]. Systems biology aims at a system-level and signal-oriented understanding of pathways by investigating 'interrelationships' (organization or structure) and 'interactions' (dynamics or behavior) of RNA transcripts, proteins, and metabolites.

2.1. Definitions of Systems Biology

There are two prevailing interpretations of what systems biology is about: 1) the integration of data, obtained from experiments at various levels and associated with the "omics family" of technologies, and 2) the dynamic interactions of gene products, proteins and cells that bring about the structure and function of cells, respectively higher levels of organization, such as for example tissue, organs, etc. The first view is more an informatics perspective, developing tools for data integration and fusion, while the second approach is motivated by data-based mathematical modeling and simulation. The first camp would often motivate their work by referring to a flood of data, while those interested in dynamic modeling of pathways are worried about the lack of quantitative, sufficiently rich data sets. It is only natural that researchers, in their quest for research funds, develop an unexpected interest in new and emerging areas of research. While systems biology covers a broad spectrum of problems in the life sciences, to pass a test for a systems biology approach one must always be able to explain how the work relates to systems theory, specifically dynamic systems theory. The term 'systems' in "Systems Biology"is, since the 1960s, associated with dynamic interactions, mathematical modeling, and simulation of biological pathways and networks. Fortunately this aspect of systems biology is not up for discussion. Systems biology signals a move away from just cataloguing and molecular characterization of the components in cells, towards an understanding of the functionality and function of cellular networks. This requires more mathematical modeling than is comfortable for some scientists, and it has subsequently become common practice to replace the term 'mathematical' with 'computational' in an attempt to hide this fact.

2.2. The Systems Biology Approach

The complexity of molecular systems is fascinating and provides many interesting challenges for theoreticians with an interest in mathematical modeling and simulation. The overused term complexity is, in the context of systems biology, clearly defined as follows:

- The difficulties in dealing with many variables that are nonlinearly related in hierarchical, multilayered networks: observability,
- The difficulties in generating quantitative stimulus-response time-series data: measurability,
- The difficulties in accounting for uncertainty, arising from a lack of observability and measurability.

The aim is that Systems Biology takes Genomics and Bioinformatics towards their natural conclusion - an understanding of the function and functioning of inter- and intra-cellular networks. For this program to succeed, it is essential, that the area attracts new people for their different perspective. The emphasis is on methodologies rather than tools and technologies. Software tools are in this context only a means to an end. More important than computing power and software tools are measurement technologies and complex designs of experiments for generating data that are suitable for a systems approach. It is a well known fact from systems theory that the behavior of a dynamic system can only be understood if it is systematically perturbed. This implies that we have to

be in a position to define input signals, keep other variables constant, while observing output variables evolve over time. The need for repeated stimulus-response experiments highlights the need for a rethinking on behalf of the experimentalists. The modeling process itself is more important than the model. The discussion between the experimentalist and the theoretician, to decide which variables to measure and why, how to formally represent interactions in a mathematical form is the basis for successful interdisciplinary research in systems biology. In light of the complexity of molecular systems and the available experimental data, systems biology is the art of making the right assumptions in modeling. The modeling process and the model are to complement the biologist's reasoning - no more but no less either. Systems biology is however not 'holistic'. We cannot escape the reductionist approach that defines science. The complexity of systems in molecular and cell biology makes it necessary to focus on subsystems, study the whole through its parts, looked at in isolation. For a multi-level and multiple technologies approach the term "integrative" may be more appropriate. The current interest in 'modules' and 'motifs' of biochemical networks illustrates this. Systems biology will hopefully bring about a new era in the life sciences but this is certainly not going to happen by means of 'new age' approaches. We should be under no illusion that it would be possible to build precise and accurate models of a cell or even organs. The concept of a 'virtual cell' carries the risk of repeating the promises and failures in other areas, including for example Artificial Intelligence. The good news is that despite the complexity of these systems, successful examples of systems biology projects have already shown that it is possible to build predictive and useful models. The cell is made up of molecules, like a car is made up from plastic and metal. But a soup of molecules is no more a cell than a heap of plastic and metal is a car. To understand the functioning and function of a cell we need to know the (static) relations and understand the (dynamic) interactions among the components that constitute it.

3. Biochemical (Interaction) Networks

Cell-biological systems are composed of diverse biochemical (interaction) networks at different levels. Cells acquire their energy, carbon, and other necessary elements from their environment and use those to sustain their own biochemical networks. Among

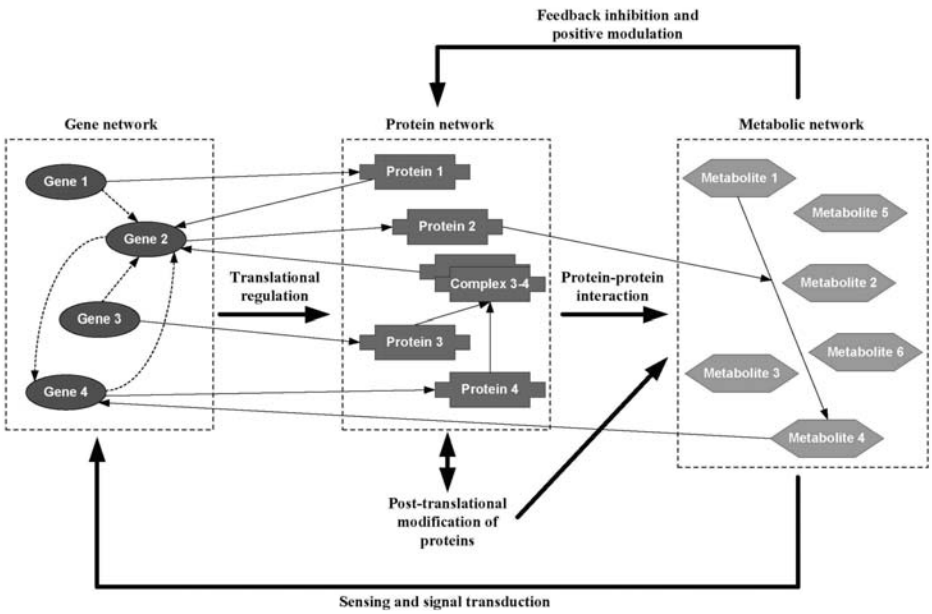

Figure 1: Illustration of the hierarchical structure of biochemical networks (adopted and modified from [11]). Understanding biological function of a cell means identifying this complex dynamic structure of the underlying biochemical network.

those networks, a metabolic network is a fundamental network of biochemical reactions, which produces the required energy for cellular functioning and decomposes/produces various molecules [11]. These metabolic networks are connected again to complex protein-protein interaction networks. These protein-protein interaction networks are further related to gene regulatory networks which contain regulatory signals determining the gene expressions [8, 9, 10]. For a full understanding, it is eventually necessary to consider all of these interactions among different molecules at different levels. Figure 1 illustrates this hierarchical structure of biochemical networks and their complicated interactions.

In terms of networks one can differentiate between static graph-theoretic networks, e.g. protein interaction maps derived from comparative genome analysis and dynamic networks. Full insight into the complex behavior of processes within a cell and in cell populations requires an understanding of both types of networks. The following section focuses on dynamic aspects.

4. Mathematical Sciences Perspectives

In mathematical modeling, one approach is to assume that the dynamic behavior of a biochemical network is best described by a set of differential equations,

$$\frac{dx}{dt} = f(x,p), \qquad x = (x_1,\mathrm{L}\ ,x_n), p = \{p_1,\mathrm{L}\ ,p_n\}, f = (f_1,\mathrm{L}\ ,f_n)$$

where a variable x_i is assigned to each network node (i.e., a gene, protein, or a modified form), and the corresponding function f_i describes how the rate of change of x_i with respect to time (left-hand side of the equation) depends on all other variables and parameters of the network. The parameters in a vector p represent any external or internal conditions such as external concentrations, rate constants, PH, temperature, etc. We can identify the dynamic behavior of a network if we know $\frac{\partial x_i}{\partial x_j}(1 \le i,j \le n, i \ne j)$. The reason is as follows. We can consider x_i as a function of $t, p, x_l (1 \le l \ne i \le n)$. In this case, we can obtain $\frac{\partial x_i}{\partial x_j}$ by regarding x_i as a function of x_j while keeping $t, p, x_l (1 \le l \le n, l \ne i, l \ne j)$ as constants. Hence $\frac{\partial x_i}{\partial x_j}$ represents the direct influence of x_j on x_i This implies that the j th node activates the i th node if $\frac{\partial x_i}{\partial x_j} > 0$, the j th node inhibits the i th node else if $\frac{\partial x_i}{\partial x_j} < 0$, and the j th node does not affect the i th node if $\frac{\partial x_i}{\partial x_j} = 0$. On the other hand, we can also identify the dynamic behavior by finding $\frac{\partial f_i}{\partial x_j}(1 \le i,j \le n)$. Since f_i is a function of $t, p, x_l (1 \le l \le n)$, we can regard f_i as a function only of x_j and can find $\frac{\partial f_i}{\partial x_j}$ while keeping $t, p, x_l (1 \le l \le n, l \ne j)$ as constants. Hence $\frac{\partial f_i}{\partial x_j}$ means the direct influence of the variation of x_j on f_i. Moreover, we note that $\frac{\partial f_i}{\partial x_j}$ also implies the direct influence of the variation of x_j on $\frac{\partial x_i}{\partial t}$ as f_i denotes the rate of variation of x_i according to time evolution, i.e., $\frac{\partial x_i}{\partial t}$. Hence we can conclude that the j th node activates the i th node if $\frac{\partial f_i}{\partial x_j} > 0$, the j th node inhibits the i th node else if $\frac{\partial f_i}{\partial x_j} < 0$, and the j th node does not affect the i th node if $\frac{\partial f_i}{\partial x_j} = 0$.

A conceptual framework of quantifying molecular interactions in cellular networks has been developed by Brown *et al.* (1997), Bruggeman *et al.* (2002), and Kholodenko *et al.* (1997) [12, 13, 14]. Two recent remarkable developments are [15] based on stationary data and [16] based on time-series data. Kholodenko *et al.* (2002) analyzed the direct effect of a small change in one network node on the activity of another node, while regarding all remaining nodes as constants [15]. Sontag *et al.* (2004) have proposed a method to describe the influence of each variable on the rate of change of each other variable by utilizing time-series data measured for parameter perturbations [16].

5. Artificial Intelligence Perspectives

As alternatives to the dynamic systems approach there are various other techniques to model dynamics, rooted in formal languages or artificial intelligence. The simplest way of representing a biochemical network is to assign binary value to each state of the network node, leading to what is called a Boolean network. In this case, the interrelationship between nodes can be described by a binary logic. For instance, if the th node is activated by the activation of the th node and the th node then we can write $x_i(t+1) = x_j(t)$ AND $x_k(t)$. Liang *et al.* (1998) developed a learning algorithm called REVEAL that is applicable to the Boolean network. This algorithm chooses the input nodes which determine the particular state of each node by computing the mutual information adopted from information theory [22]. The mutual information is used as a basis for comparing/determining the most influential adjacent network node. Shmulevich *et al.* (2002) have further extended this framework and proposed a probabilistic Boolean network in which multiple binary logic determines a particular state of a biochemical node and a characteristic probability pertains to each binary logic [25].

A Bayesian network is a kind of probabilistic graph models, representing a joint probability of variables and usually described by a directed acyclic graph. Each node of the graph denotes a variable of the biochemical network and the structure of the graph indicates the conditional independence of the variables. In addition, each arrow represents the probabilistic dependence between the nodes. Friedman *et al.* (2000) have applied the Bayesian network approach to investigate the interaction of genes based on microarray experiments [17]. Hwang *et al.* (2002) have further developed an efficient structure-learning algorithm and a data space dimensional reduction method to resolve the difficulties in machine learning of the Bayesian network structure and in dealing with the dimensionality problem of the huge data sets of a gene network [19]. Kim *et al.* (2003) have proposed an efficient dynamic Bayesian network approach to reverse engineer a gene network based on time-series microarray data [21].

There is another formal framework called an S-system which can describe the state transition and the interaction between biochemical entities from the time-series measurement [26]. Since the S-system is composed of well-structured nonlinear differential equations, it can be easily turned into a graph model representing the interaction of the biochemical entities. However, there are too many parameters to be estimated in this model. Kikuchi *et al.* (2003) have applied the evolutionay programing to efficiently estimate those parameters of the S-system and Maki *et al.* (2001) have studied identifying a large gene interaction network by integrating the Boolean network and the S-system approaches [20, 23].

Recently, new attempts have been made to investigate the interactions of biochemical entities such genes, proteins, metabolites, *etc.* by extracting relevant information from the accumulated biological literature through text mining. To this end, some artificial intelligence methods such as natural language processing and information retrieval have been used (Rzhetsky *et al.*, 2004) [24].

6. Statistical Physics Perspectives

Statistical physicists have long been interested in the emergent properties of interacting many-body systems. A qualitative change in the properties of a system usually occurs abruptly as the topology of the embedded space or the pattern of an interaction varies. It was not until recently, however, before they turned their attention to more generic architectures of complex networks. Such an interest has also been boosted by the discovery of the ubiquity of a nontrivial pattern which is strikingly different from the traditionally-assumed “lattice” structure. Networks in diverse contexts, from the internet through the social network to the cellular networks, share common characteristics. For example, they all exhibit the power-law degree distributions, to which the notion of a “scale-free network” has been attributed (Barabasi and Albert, 1999) [29].

Such then an unexpected structure affects the dynamic properties of a system greatly. To a large extent, a form is followed by a function. Studies have been carried out to see how the property of the well-known statistical physical processes would change by the presence of a power-law degree distribution. These include the percolation problem (Cohen *et al*., 2002), the epidemic spreading (Pastor-Satorras and Vespignani, 2001), the self-organized criticality (Goh *et al*. 2003), *etc* [31, 35, 33]. Roughly speaking, it can be said that the dynamic property of a scale-free network is governed mostly by the presence of the hub, the node with a large degree, in the network. More precisely, it is related to the fluctuation in the degrees of nodes, which diverges as the hub develops. For example, in epidemic spreading, the presence of the hub greatly accelerates the spreading of epidemic over the network and lowers the epidemic thresholds even to zero. More biologically motivated problems like Kauffman's Boolean network (Aldana 2003) or the synchronization transition of coupled oscillators (Strogatz 2003) have also been studied on top of scale-free networks [27, 36]. For instance, Aldana showed that the Boolean network tends to exhibit a chaotic trajectory as the heterogeneity of a network increases.

To be more specific to biochemical networks, the studies so far have been related mostly to uncover the topological organization of various types of cellular networks, such as metabolic networks, protein interaction networks, and transcriptional regulatory networks. Almost all of the investigated has something to do with the scale-free network [30]. Only recently we have gone further. For example, Almaas *et al*. showed from an *in silico* analysis that the flux levels across a metabolic network are also highly heterogeneous, as they also exhibit the power-law distribution [28]. Networks are thought to be made of network motifs [34]. The motifs sometimes aggregate to form a motif-cluster [32]. This means that a challenge is now upon for us. We want to figure out the overall function of a system from its basic functional contents, network motifs, by understanding the effect of the pattern of their aggregation, which will eventually build up the whole.

7. Discussion

As Mesarovic mentioned in (Mesarovic *et al*., 2004), the life sciences could become a science with a conceptual structure and logical coherence, and the field of systems biology has reemerged towards this end [39]. There is a whole range of views on what systems biology is all about, but the essence is to provide a conceptual framework within which complex cell biological functions, cell-cell interactions and their effects on the physiology of an organism can be understood. The core of understanding in systems biology depends on the search for organizing principles of complex biological phenomena, which underlie biochemical networks. This paper reviewed various approaches for investigating these organizing principles by unraveling the dynamic structure of a biochemical network for this purpose. There are many more approaches competing for the attention of the biologists and the reader is referred to the various special issues and survey papers that have been published, including for example [37, 38]

In the context of post-genome technologies, the key challenges in systems biology can be summarized as dealing with 'complexity' (defined by a large number of variables and nonlinear interactions) and 'uncertainty' (due to the difficulties in observing cellular dynamics). Modeling the interaction of phenomena that happen on a wide range of scales in space, and time, i.e., organized complexity, is therefore a primary post-genomic challenge. The data we currently have available on the other hand, do not permit the use of well established engineering tools for parametric system identification. Systems biology subsequently relies on improved technologies to allow the generation of quantitative, sufficiently long, time-series from stimulus-response perturbation experiments. Without being too pessimistic, one might imagine mathematical modeling and simulation of whole cells have the same fate as weather forecasting: regardless of the computer power and time available, the predictions remain uncertain. However, even if we will never be able to build accurate predictive models of cellular or genetic systems, systems thinking and the modeling process itself will prove valuable to the biologist, helping him/her to identify which variables to measure and why. In fact, a common engineering experience is that we learn most from those models that fail. The quest for precision is analogous to

the quest for certainty and both precision and absolute certainty are impossible to attain in understanding cellular dynamics, at present if not in general. Experience shows that even dramatically simplified models, based on many assumptions, can still capture essential phenomena and thereby support hypothesis testing and experimental design.

Acknowledgment

The preparation of this article benefited from discussions with Prof. S.-M. Choo, Dr. K.-I. Goh, D.-Y. Cho, and H.-S. Choi. This work was supported by a grant from the Korea Ministry of Science and Technology (Korean Systems Biology Research Grant, M10309000006-03B5000-00211). The work of Olaf Wolkenhauer was supported, in part, by a grant from the UK Department for the Environment, Food and Rural Affairs (DEFRA).

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