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Simulation of biochemical networks - Cellular networks as dynamic control systems

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Abstract— **It has been appreciated for at least a hundred years that biological organisms contain control systems that enable them to adapt to a changing environment and adjust their internal systems when they need to proliferate. Even so, we have little understanding of the role that many of the control systems play. It's only in recent years that mainstream science has begun to study biological systems qualitatively and to look specifically at dynamical responses. As a result it might be possible that future cancer therapies will operate by manipulating the control systems that have gone awry during uncontrolled proliferation. This is a long term goal because it would require a mind shift in the way some biologists approach such problems. In this short paper I describe some of the main control elements found in biological systems and illustrate their use in biological networks. In addition I discuss some of the strategies that one can use to build computational models.**

I. INTRODUCTION

One of the striking features of biological systems is the multitude of control systems that lace many cellular networks. Feedback and feed-forward loops are found at all levels of cellular organization, including metabolism, protein and genetic networks. In the majority of cases we have little understanding of their role. In most studies the existence of a control loop in a system is only given cursory consideration and in modern high throughput studies control systems are usually entirely ignored. The reasons for this are partly down to the difficulty, at least historically, in making the required measurements but is also related to the way biologists view biological systems as static structures. However, in recent years new techniques, such as fluorescence tagging, have been devised that permit real-time measurements of dynamics at the molecular level. In addition, these studies have been carried out on single cells which has revealed a unexpected degree of rich dynamics [16].

If we take the reasonable view, that biological networks can be treated as control systems then questions related to the onset of cancer can be reduced to questions of how cellular control systems misbehave in the disease state. Although the onset and development of cancer is a complex and multi-factor problem, uncontrolled proliferation can be attributed in many cases to a failure of some control aspect inside the cell. If we could understand the dynamics of cellular systems from a control perspective, it might be possible to devise strategies to correct the error.

Understanding biological systems is essentially a reverse engineering problem. It is like trying to understanding an alien technology; we know that the technology *probably* uses principles that are employed in our own technological devices. However there are many things we don't know; we do not know in detail the properties of the component parts, assuming we can identify them; we would also find it difficult to make measurements of the internal state and although we know gross input/output responses we do not understand how the internal mechanisms generate the transfer relationship. Biologists, however, have one great advantage over those who would reverse engineer an alien artifact. Presumably we would only have one artifact in our possession and thus we would have to be very careful in how we study the artifact lest we would destroy the only copy we have. Biologists on the other hand have the advantage of access to potentially millions of copies of an organism which means that a biologist can destroy the system under study knowing that another one is just a replication cycle away.

In the short paper I will cover some aspects of control systems in relation to biological networks.

II. COMPUTATIONAL CAPACITY OF CELLULAR NETWORKS

There are arguably three fundamental control related devices repeatedly used in biological networks, these include:

- Feedback and Feed-forward control loops
- Covalent Modification Cycles
- Allostery, including gene-protein interactions

A. Feedback and Feed-forward Networks

Feedback and feed-forward mechanisms are the two basic control motifs found in almost all biochemical systems. They come in two flavors, negative and positive. The most well known of these is the infamous negative feedback loop, first discovered in metabolic systems by Umbarger [27] and Yates and Pardee [32] in the 1950s. The most cited property that negative feedback confers is homeostasis, that is negative feedback stabilizes an end product in the face of varying demand. However other less appreciated properties include immunity to noise generated within the feedback loop, linearization of the input/output response and a reduced sensitivity to changes at the input. Some or all of these properties may have biological significance.

Whereas negative feedback tends to have a stabilizing effect, positive feedback has the opposite [6]. There are now a number of examples cited in the literature where positive feedback is used to generate two stable steady states, usually designated high and low. Such systems are called bistable. The two states tend to be stable and resist changing from

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one state to the other. As a result, positive feedback can be used to create decision circuitry where a definite off and on state is desired [7].

Feedback mechanisms are also employed to generate oscillatory dynamics. Negative feedback can be used to generate oscillations simply by ensuring that the delay between signal and action is long enough and that the feedback gain is sufficiently high (Figure 1). Alternatively, a positive and a negative feedback can be combined to form a relaxation oscillator where the negative feedback drives the bistable subnetwork from one state to another (Figure 2).

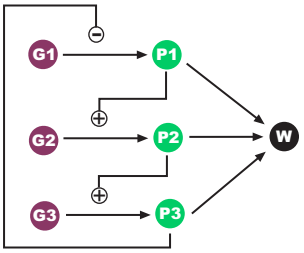

Fig. 1. Feedback oscillator using negative feedback. G1 to G3 are genes, P1 to P3 are proteins transcribed from their respective genes. w indicates a waste node. Each protein can activate or inhibit the production of another protein, indicated by positive and negative interaction loops.

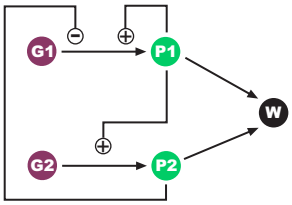

Fig. 2. Relaxation oscillator employing positive feedback. G1 and G2 are genes, P1 and P2 are proteins transcribed from their respective genes. w indicates a waste node. Each protein can activate or inhibit the production of another protein, indicated by positive and negative interaction loops.

Feed-forward networks have a different set of properties to the feedback systems. Although such motifs have been discussed in relation to neural systems for many years, it is only recently that feed-forward networks have been of interest to the cellular network community [17], [23]. Feed-forward networks are flexible and occur frequently in genetic networks (apparently more frequently that feedback loops). A negative feed-forward (sometimes termed an incoherent type 1 network) can act as an amplitude filter (Figure 3); for a given input concentration range the response of the network is maximal. Given such a property, numerous other devices can be constructed including event generators and homeostatic devices. Such versatility may explain the frequent occurrence of such networks in real biological systems.

B. Covalent Modification Cycles

One of the most sticking features of protein-protein networks is the prevalence of covalent modification cycles. Such cycles form the backbone of many protein-protein networks, particularly in higher organisms. Covalent modification permits an organism to use one protein in multiple

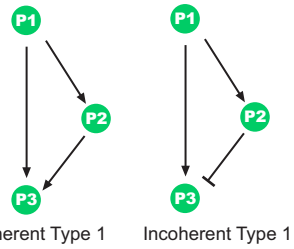

Fig. 3. Two examples of feed-forward motifs [17], [23]. The symbol P1 to P3 denote a particular biological process, usually the expression of a gene.

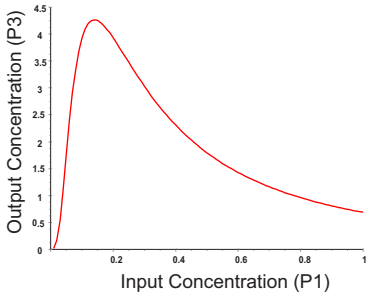

Fig. 4. Response curve for the incoherent Type 1 feed-forward motif. The x axis marks the concentration of the input signal (P3 in Figure 3) and the y axis the output of the pathway, (P1 in Figure 3)

states and thereby effectively increase the number of proteins and thus the network complexity. Covalent modification is often implemented using phosphorylation as the species tag but other approaches are employed, including methylation, acetylation and uridylation.

It has been appreciated for many decades [8] that a covalent cycle can show threshold properties and there is now ample experimental evidence to support this view [2]. When a covalent cycle acts as a threshold device it is behaving in a manner similar way to an operational amplifier [11]. Like an operational amplifier the behavior of covalent cycles can be modified by introducing feedback loops. Thus in principle, one could devise summers, integrators, differentiators etc. from covalent cycles [21]. In addition, with sufficient feedback and delay it is possible to generate oscillatory dynamics[13] and with positive feedback it is possible to generate bistable systems [7]. There are also subtle sequestration effects that can greatly influence dynamic behavior, which on the whole have been largely unexplored [18].

C. Allostery/Gene-Protein Interactions

Allostery and it's close companion, cooperativity, are found in many proteins. One of the main characteristics that such proteins have is a sigmoid response to effectors. Similar behavior can also be found in gene-protein interactions. Gene-Protein interactions can generate even more varied behaviors including logic gates and complex analog responses. The key aspect that allostery provides is linear high gain (Figure 5). Without cooperativity, the response of an allosteric enzyme would be nonlinear and low gain, neither desirable characteristics for a control mechanism. The gain generated by an allosteric enzyme is used to generate the

necessary feedback strength while the linearity is required to generate a proportional response difference between signal and response.

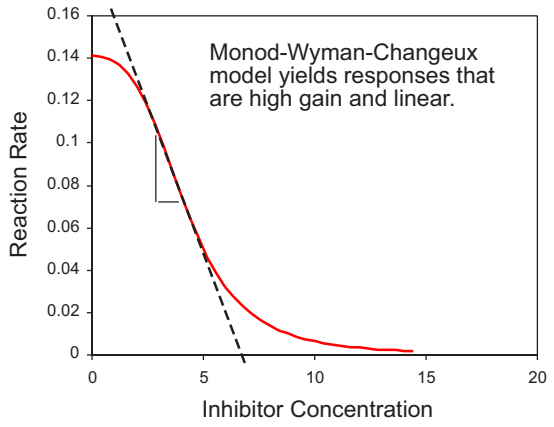

Fig. 5. Response curve for an allosteric model based on the MWC (Monod, Wyman and Changeux [20]) mechanism. The graph shows the reaction rate versus the concentration of inhibitor. Note that, unlike other kinds of inhibition, allostery coupled with cooperativity provides a highly linear response with a large gain (slope).

III. Modularity of Networks

The apparent complexity of biological networks is abundantly clear when we view the many network graphs now available as posters or diagrams in text books. However, we perceive the network as being complex because we choose to view the entire network in all it's intricate detail and are inevitably overwhelmed by the number of components and connections. The engineering disciplines take a different approach, rather than attempt to view the entire network, engineers divide networks into modules, such modules are further divided as necessary, resulting in a hierarchy of functional descriptions. It is this separation of behavioral levels that allows engineers to comprehend and build highly complex information processing systems. In biology, we can use a similar strategy by breaking biological networks into simpler and more manageable modules. The process of understanding complex biological networks then involves describing and locating functional modules in a larger network.

The difficult question then arises, what is a functional module? There have been numerous discussions of this issue in the literature [9], [26], [31] and a number of common themes have emerged. A key idea is replacement, where a module can be replaced without disturbing the rest of the system behavior. With replacement comes the notion of an interface, where a module has a defined interface which is the point of contact between the module and the rest of the system. Finally, the number of contact points at a module interface will often be smaller than the number of interactions internal to the module. This latter aspect is of interest because it has been used to uncover modules in complex networks. In particular, a common metric [4] used to uncover topological modularity in networks is based on this very idea.

All the techniques employed today to discover modularity are based on the notion of topological modularity, that is finding topological patterns that might suggest a modular structure. Although such work is a good starting point, what we really want to seek is *functional* modularity. Unfortunately this is a much more difficult problem and no clear solutions currently exist.

To highlight the problem with delineating functional modular structure consider the example of the simplest network, a simple linear chain of reactions (Figure 6).

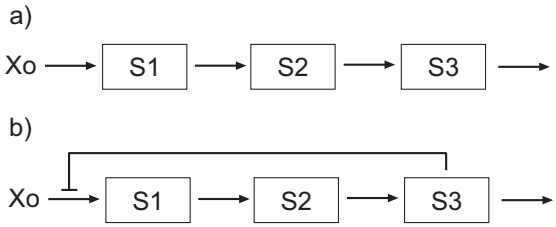

Fig. 6. Two figures, a) and b) showing the conventional way to depict pathways. S1 to S3 are molecular species, the arrows between the species represent reactions. Xo is a fixed boundary species. The second pathway (b) has a feedback mechanism from species S3 to the first reaction.

We can write down the differential equations for this system as:

$$\begin{aligned}
\frac{dS1}{dt} &= v_o(Xo, S1, S3) - v_1(S1, S2) \\
\frac{dS2}{dt} &= v_1(S1, S2) - v_2(S2, S3) \\
\frac{dS3}{dt} &= v_2(S2, S3) - v_3(S3)
\end{aligned}$$

where v_o to v_3 indicate the reaction rate for the four reactions in the pathway. Each reaction rate is described as a function of one or more species, eg $v_1(S1, S2)$ means that the reaction rate v_1 (second reaction) is a function of S_1 and S_2.

The $S3$ in the first equation is absent in the pathway which has no feedback control. By examining the functional dependencies we can redraw the conventional map to reveal a much greater degree of control in the pathway.

Although redrawing the maps in this ways reveals many more interactions, one can also see obvious regularity in the control structure. It might be possible for example to use computer based algorithms to search for specific control patterns, thus rather than attempt to identify topological patterns we could instead search for control patterns which in turn would presumable indicate functional properties.

The question of identifying functional modularity in biological networks is an unresolved question and is, from my perspective, one of the most challenging and important questions in systems biology. If we believe that by manipulating the control systems in a cancer cells we can restore such cells

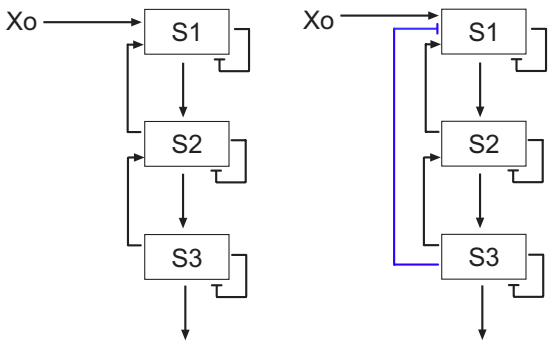

Fig. 7. Redrawing a conventional pathway diagram to illustrate the detailed control systems present even in the simplest pathway. Left-hand pathway corresponds to Figure 6a and right-hand pathway to Figure 6b.

to their normal state then an understanding of the functional structure of networks is important. In addition, once we understand the functional structure of cellular networks we can also use this information to greatly increase the reliability of our computational models.

IV. Building Models

I have so far discussed issues related to how biological networks are controlled, however an obvious question is how does one generate a model in the first place?

The starting point for any model is a topological map of the known interactions. Often such topological maps will take the form shown in Figure 8.

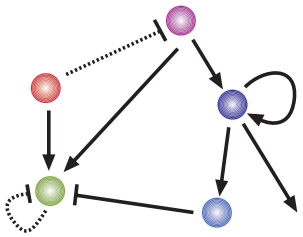

Fig. 8. A typical network interaction map as published in the literature. The nodes on the map usual indicate protein species of a gene expression process. Arrows are used to indicate whether one process activates or inhibits another. The dotted lines indicate unknown interactions that might be present.

Unfortunately such interaction maps cannot be used to derive a mathematical model because they are incomplete, in particular they lack any information related to stoichiometry and miss many important steps in the network. Instead one must deal with stoichiometric maps which explicitly indicate the transformations that occur in the model, such as gene expression, protein turnover, covalent modifications and metabolic processes. Control actions are represented either as binding reactions which sequester important intermediates or by explicitly including the control function as a term in a rate law. I have converted the interaction map in Figure 8 into the stoichiometric map shown in Figure 9. In this form we can generate a mathematical model. The essential requirement is that one should be able to discern consumption and production rates around each species, something that cannot be done with interaction maps.

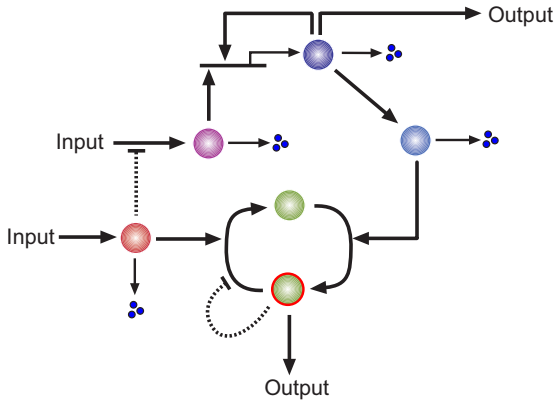

Fig. 9. Redrawn interaction map to reveal hidden reaction steps and make explicit the stoichiometric nature of the network

Once a stoichiometric map has been drawn, each species is converted into a differential equation describing the net balance between production and consumption of that species. This representation can be described using the equation

$$\frac{d\boldsymbol{S}}{dt} = \boldsymbol{N}\boldsymbol{v}$$

where $\boldsymbol{N}$ is the stoichiometry matrix and $\boldsymbol{v}$ the rate vector. The stoichiometric map is described by the $\boldsymbol{N}$ term while the kinetics are described by the $\boldsymbol{v}$ term. In many cases, particularly with genetic and protein networks, some interactions may be missing and part of the process of model building is to try and fill the gaps.

Once a satisfactory stoichiometry map is available, attention can turn to defining the kinetic laws at each reaction step. This is where most of the difficulty arises since in many cases the kinetics constants will be poorly known or not known at all. The first decision to make however is to decide on the types of kinetic laws to use. A modeler confronted with such a decision has a number of possibilities to choose from. The list below details the most common rate laws that can be employed.

- Mass-Action Kinetics, used when very little data is available or when it is known that the reaction is governed by simple mass-action.

  $$v = k \prod S_i$$

  where k is the mass-action rate constant and S_i the concentration of a species. v is the reaction rate.
- Michaelis-Menten. The basic irreversible enzyme rate law is useful for many situations. However care has to be taken when enzyme and substrate concentrations are comparable. Such cases can lead to significant sequestration effects. This is a particular problem when modeling protein-protein networks and in such situations, it is better to derive custom Michaelis-Menten rate laws that takes into account such factors, see [18] for an example.

  $$v = \frac{V_{max} S}{K_m + S}$$

where Vmax is the maximal velocity, S the species concentration and K_m the Michaelis constant.

- Surprisingly unknown to many biologists, the reversible Michaelis-Menten is the preferred equation over the simple irreversible Michaelis-Menten rate law. Unless it is known with some certainty that the reaction is irreversible, the default choice should be this rate law. As discussed earlier, reversibility is an important route by which perturbation information is transmitted back up a pathway. Metabolic pathway models benefit considerably from using the reversible form.

$$v = \frac{V_{max}/K_{M_1}\left(S_1 - S_2/K_{eq}\right)}{1 + S_1/K_{m_1} + S_2/K_{m_2}}$$

  where the symbols in the equation have a similar meaning to the previous equation. K_{eq} is the equilibrium constant for the reaction.
- The Hill equation is often the work horse for generating sigmoidal responses and as a first approximation, it is often used to model gene-protein interactions, particularly for simple cases. The most important variants include the addition of repression and activation. Activation:

$$v = V_{max}\frac{S^n}{K^n + S^n}$$

  Repression:

$$v = \frac{V_{max}}{K^n + S^n}$$

  n is the Hill coefficient, the remaining symbols are as described previously.
- Much more elaborate is the reversible Hill equation by Hofmeyr and Cornish-Bowden [10] and is a good substitute for the Monod, Wyman, Changeux Model [20] when dealing with metabolic pathways.

$$v = \frac{V_f S_1/K_{M_1}\left(1 - \Gamma/K_{eq}\right)\left(S_1/K_{M_1} + S_2/K_{M_1}\right)^{n-1}}{1 + \left(S_1/K_{m_1} + S_2/K_{m_2}\right)^n \frac{1+(X/K_x)^n}{1+\alpha(X/K_x)^n}}$$

  The symbols are as described previously. Γ is the mass action ratio and V_f is the forward maximal velocity.
- For more complex gene-protein interactions the recommendation is to derive custom rate laws using the rapid-equilibrium methodology. This approach was popularized by the work of Shea and Ackers [1]. They modified the traditional rapid-equilibrium method by relating the equilibrium constants of binding steps to the binding energy (in the form of ΔG) between the transcriptional factors (or RNA Polymerase) and the DNA binding sites.
- Finally, in this short list, one should also mention the linlog approximation. In building metabolic models, the linlog approximation is an extremely useful equation to use. It requires only minimal information and has been shown to provide a very good approximation to Michaelien type responses, the reader is referred to [15] for more details.

$$\frac{v}{J_o} = \left[\frac{e}{e_o}\right]\left(1 + \sum \varepsilon \frac{x}{x_o}\right)$$

  where J_o is the reference velocity, e_o the reference activity of the enzyme, x_o is the reference species concentration and ε is the elasticity coefficient. Note that the summation refers to the number of interactions that could affect the enzyme.

The above list is obviously by no means complete, in particular the rapid-equilibrium and steady-state assumptions can be used to derive many different rate laws, including different kinds of inhibition and activation. The book by Segel [22] illustrates many more kinetic schemes.

With the topology and reaction kinetics decided, decisions have to be made on what values to give the many constants in the model. These constants will include terms such as Vmax's, Km's, Hill coefficients, binding constants etc. In many cases, these constants will not be known. For metabolic models the situation is better because in the days when metabolic pathways were being elucidated, characterizing the enzymes of the pathway was considered an important part of the work, as a result there is a rich supply of data concerning kinetic data for metabolic enzymes. Some would argue that given the *in vitro* nature of this data, and in many cases the unrealistic conditions under which the data was measured, this data is highly suspect. However, such data has proved to be useful, at least for giving the basic magnitudes of the various constants. In addition, it has been found that biological systems tend to be remarkably robust and in many cases, accurate determinations are not important except for a small number of constants. One strategy is to use a sensitivity analysis on the initial model to determine which constants are the most important in determining model behavior, in this way the modeler can focus on obtaining accurate values for the most important constants of the model.

For protein-protein networks the problem is more difficult, largely because the kinetic assays are much more difficult to carry out. However, even here, due to the general robustness of networks, one can often develop models that qualitatively reproduce the expected behavior [3]. This brings us to another point, in the initial development of a model it might be sufficient to develop a model that can qualitatively reproduce a particular biological phenomenon. The reason why this is a credible approach is because ultimately a model is only useful if it can predict behavior not yet observed and qualitative models can predict qualitative behavior.

Another strategy used by some authors [3], [5] when building larger models is to modularize a model into distinct functional parts and to focus on getting the functional part to work before moving on to the whole model. If the functional parts are expected to have particular behaviors such as bistability or oscillatory dynamics, tools such as the bifurcation discovery tool [4] can be use to place the model in the correct parameter region before work commences on fine tuning the parameter values.

Developing models capable of quantitative prediction is more difficult. One approach is to adjust the constants in a

model so that the model predicts gross phenotypic behavior. This approach has proved particulary useful in modeling the cell cycle where one can measure times between particular events and the size of cells at particular times during the cell cycle. Such properties can be easily measured in a model, moreover, there are many known mutants which affect these properties, for example by lengthening or shortening times. Such mutants can be induced in the model and the effects observed. John Tyson and his colleagues [24] have used this technique extensively to build a high realistic and accurate model of the yeast cell cycle [25]. In addition the Tyson group has also developed software that automates the process of validating the many mutants and their effects on the model [28].

Another approach requires more detailed measurements of the behavior of the system in time. Such measurements should attempt to include high resolution time course data of selected species in the model. In the past this was difficult if not impossible to do, however with the rapid development in fluorescence tagging (the ubiquitous GFP tag) in recent years and the focus on single cell measurements it is actually becoming possible to make such measurements. Much of this impetus is coming from the synthetic biology community where high resolution measurements are required to confirm the functional properties of the synthetic network [12].

With time course data one can employ parameter fitting techniques. This method involves fitting the set of model differential equations to time course data. The idea is that the constants in the equations are adjusted until the time evolution of the model matches the experimentally determined time course behavior. There is a considerable literature on this topic and for the uninitiated the paper by Mendes [19] is recommended. For a more detailed overview, the paper by Kremling *et. al.* [14] covers additional topics.

In the final analysis, much depends on the experience of the model builder. After building a number of models one will often come to realize what values parameters should have in order to elicit a certain behavior. In addition, with enough experience it is also possible to suggest missing interactions. Such predictions are of course one of the main reasons for building models. A critical factor in the success of a model is the availability of suitable data but what is very important is that a model should drive the experimental agenda, not the other way round. A number of recent projects have failed in this respect because the modeling was secondary to the experimentation and as a result they did not to live up to expectations. This approach is new to many biologists and the transition is certainly difficult.

V. Conclusions

The development of Systems Biology in mainstream science is still early. On the whole, we still have a very poor understanding of how most, if not all, biological networks operate at the functional level. Even glycolysis, the first documented pathway is not fully understood, we do not understand for example the role for many of the feedback and feed-forward loops in glycolysis. Moreover, although we have detailed molecular structures for all the glycolytic enzymes we only have adequate kinetic descriptions for a few. Most of our kinetic information we have dates from the 1960s and 70s when it was popular to characterize enzymes kinetically. The lack of data is not a technological problem but a sociological one. Fortunately, the possession of detailed kinetic information for every reaction step in a model is not critical and for many steps it can be sufficient to use some form of kinetic approximation [29]. One aspect that is in the modelers favor is that many systems appear to be relatively robust to parameter changes (See [30] for a detailed and accessible discussion of this topic). I would therefore suggest that the lack of kinetic data may not be the most important impediment to building successful models even though some authors claim it to be. I believe what is more important than kinetic data is the possession of high resolution measurements of state variables, that is real-time measurements of protein and gene activity.

There are encouraging signs in the research community, in particular the new field called synthetic biology may point the way forward [12]. Rather than catalog parts and connections, synthetic biology attempts to reconstruct networks and to study their dynamics in detail, experimentally and by building computational models. Technological advances, particulary in cell counting techniques and light microscopy enable synthetic biologists to collect large amounts of *high* resolution data on a *small* number of observables. This is in contrast to contemporary high-throughput approaches which collect *low* resolution data on *many* observables. I think the most encouraging result so far to come from synthetic biology [12] is that our basic understanding of physical biochemistry appears to be largely correct, if it wasn't the computational models would not match so well the experimental results. Some authors claim that building computational models is a futile effort given our current understanding of biological processes, however this is patently not true given the results from synthetic biology. If there is one rule of thumb to running a successful modeling project, it is that the modeling should drive the experimental agenda, and not the other way round. Is is only by forcing the modeling process to the top of the pile that one comes to realize what data are missing and what predictions need to be tested. By a step by step process, from model to experiment and back again, will the model converge on to the biological reality.

What does this have to do with cancer research, probably a lot more than one might realize. The systems that control cell proliferation appear to incorporate devices and control strategies that can be found in our own technological systems. The cells monitor their internal and external environments and decide from the multitude of inputs the next course of action, such decisions are computed through protein networks. A breakdown of the decision process is clearly bad for the cell and is something biological cells appear to have attempted to mitigate since in the event of excessive DNA damage for example, many cells will initiate apoptosis to protect the organism. Sometimes the failsafes do not operate

and cell proliferation can result. To understand the control mechanisms at work and to find ways to intervene that can return control back to the normal state is clearly something we would all like to see. However there needs to be a change in the mind set in order to achieve this. The most promising avenue I feel is work carried out by researchers on the time course dynamics of proteins in single cells [16]. Such work has revealed an unexpected rich variety of dynamical behavior. This work is reminiscent of the synthetic biology community in that single cell studies focus on measuring at high resolution, a few observables and then attempting to relate these measurements back to computational models. It is these kinds of studies that will reap the most benefit to understanding uncontrolled proliferation at the protein network level. Ultimately this will also lead to reliable computational models which in turn will be indispensable to devising new therapeutic approaches.

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