Symbolic Systems Biology: Hybrid Modeling and Analysis of Biological Networks*

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Abstract. How do living cells compute and control themselves, and communicate with their environment? We describe the modeling and analysis of dynamic and reactive biological systems involving both discrete and continuous behaviors, to help begin to answer that question. Continuous components arise as differential equations specifying how the concentrations of various molecular species evolve over time. Discrete components of models of biological systems arise from state transitions (e.g., from healthy to abnormal states), abstractions and approximations, nonlinear effects, and the presence of inherently discrete processes, often observed in systems governed by one or a few molecules. Once a hybrid model is obtained, analysis techniques such as those described in this and previous HSCC workshops can be usefully applied to help uncover structure in the dynamics of biological systems of interest.

1 Introduction

The completion of the Human Genome project, the initial elucidation of many important biological pathways, and the deluge of biological data becoming available provide exciting opportunities for computer-assisted analysis of biological systems, with huge potential future impact. Biology is now an information science, with many different types of information: the one-dimensional structure of DNA encoded genes, the three-dimensional structures of proteins, and the unimaginably high dimensional dynamics of biological network interaction. It is the latter network interaction topic that we address here. Many databases, standard modeling languages, and tools are now becoming available for biological information having to do with network effects. In particular, the Biological Simulation Program for Intra-Cellular Evaluation (BioSPICE) is an open source development movement that is creating computational models, tools, and infrastructure to help deal with modeling and analysis of complex biological systems. Begun in 2002, BioSPICE seeks to explore, develop, and exploit both the role computation may play in biology and the role biology may play in computation [15, 21, 8].

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However, despite the exponentially expanding oceans of biological data becoming available, to understand how cells compute and control themselves, we must build accurate models that represent the aspects salient to the questions of biologists. Even relatively simple prokaryotic cells, let alone more complex eukaryotic (e.g., human) cells, are so complex that we must aggressively abstract the models to enable more complete, deep, and scalable analysis, and to present results to biological domain experts.

The construction of mathematical models for biological processes is central to the science of bioinformatics and computational biology, but the inherent complexity of biological systems is daunting. Biological processes exhibit dynamics that range over a very wide time scale and contain stochastic components and sometimes discrete components as well. Sigmoidal nonlinearities are commonly observed in biological data correlation, and a wide class of such functions is used in the resulting models. Biological process operate at widely disparate time and spatial scales, spanning 12 or more orders of magnitude (from single cell to entire organism). A complete model of a biological process is complex and poses a challenge for simulation and analysis.

Genetic regulatory networks that work inside a cell form one class of biological system. Such networks are responsible for various kinds of cellular behaviors, for instance, recording, computing, and reacting to changes in the environment. Behavior is controlled through complex interaction between various protein concentrations that are regulated by transcription of various genes, and which, in turn, positively or negatively influence transcription of other genes, thus resulting in complex interwoven networks of control. At a much larger scale, metabolism can be studied at the level of the whole human body. For example, glucose metabolism can be modeled to determine the blood glucose concentration in human tissues. In these cases, a phenomenological model is constructed using tissue- and organ-level concentrations as basic state variables.

The old but recently burgeoning field of systems biology explores the quantitative study of biological processes as whole systems instead of isolated parts [30, 22, 24, 17, 19]. Biological subsystems interact with one another to perform sophisticated biological functions, and a systems-level view is necessary to understand the complex dynamics that underlie physiology in normal and diseased states. Systems biology research has focused on quantitative stochastic or differential equation models of biological systems.

Mathematical models of biological processes are often constructed by generating equations describing the physical laws that govern the system dynamics. The obtained model is tuned by determining free parameters and unknown rate constants using experimental data. However, this is not always possible, as quantitative experimental data is plagued with high levels of noise, and precise rates of reactions are for the most part unknown to science. Even when some rate constants have been inferred using algorithms for determining minimal error curve fits for available data points, the resulting model is just a "representative" that best matches all the available data. The actual value of the parameter or rate constant is possibly stochastic in a given range, so there is a danger of overfitting quantitative models to the data, resulting in inaccurate predictions that are highly sensitive to small perturbations in input data. Moreover, the number of different state variables can grow quite large. Too many variables representing different molecular species involved in various compartments can adversely affect our ability to subsequently simulate and analyze the model. To further complicate matters, assumptions about homogeneity and the presence of a large number of molecules often break down at the cellular and cellular compartment levels. This means that mathematical models based on such assumptions cannot be expected to be accurate [2].

An alternative approach is to seek completely qualitative, rather than quantitative models of biological systems. Some examples of this approach include the high-quality curation and analysis of qualitative metabolic pathway information [18, 29], symbolic analysis including term rewriting and model checking of curated pathway models [6, 32], and other logical modeling approaches [40, 28]. However, one need not focus on completely logical or symbolic mathematical modeling of biological systems. We can use hybrid systems as an underlying formalism for modeling and analyzing biological systems. We refer to the qualitative or hybrid qualitative-quantitative modeling and analysis of biological systems as *Symbolic Systems Biology*.

2 Hybrid Models

Hybrid systems are mathematical models obtained by formally combining continuous dynamical systems with discrete transition systems.

Continuous component

In a hybrid system, the continuous dynamics of time-varying variables are given using differential equations. In models from biology, the differential equations specify how the concentrations of various molecular species evolve over time. These differential equations are obtained using standard physical laws, such as the law of mass action and the law of mass conservation.

For example, consider the case when a species X reacts (reversibly) with another species Y to form a complex XY. Schematically, this is represented by

$$X + Y \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} XY$$

where k_1 and k_{-1} are the reaction rates for the forward and backward reactions respectively. If x, y, and z denote the concentrations of X, Y, and XY respectively, then using the law of mass action, which states that the rate of a reaction is proportional to the products of the concentrations of the reactants, we get a system of three differential equations:

$$\begin{aligned}
\dot{x} &= -k_1 xy + k_{-1} z \\
\dot{y} &= -k_1 xy + k_{-1} z \\
\dot{z} &= k_1 xy - k_{-1} z
\end{aligned}$$

If a species, say X, participates in more than one reaction, say

$$X + Y_i \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} XY_i, \qquad \text{for } j = 1, \ldots, l,$$

then its rate equation is obtained by collecting terms from each reaction in which it participates. Adding a source and a sink term, this becomes

$$\dot{x} = \sum_{j=1}^{l} k_j^{-1} z_j - \sum_{j=1}^{l} k_j y_j x + r_{src} - r_{sink}, \tag{1}$$

where z_j represents the concentration of the complex XY_j, r_{src} is the rate of production of X, and r_{sink} is the rate of utilization of X (independent of the reactions that have been accounted for explicitly). For example, if X were a protein, then the effect of the production of X by transcription would contribute a source term and its decay by proteolysis would contribute a sink term.

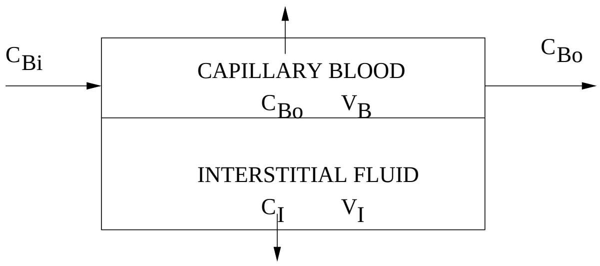

Fig. 1. A physiologic compartment

In the example of modeling the blood glucose in human body [31], consider a typical physiologic compartment shown in Figure 1. The mass balances for this compartment can be written as

$$\begin{aligned} V_B \dot{C_{Bo}} &= Q_B(C_{Bi} - C_{Bo}) + PA(C_I - C_{Bo}) - r_{RBC} \\ V_I \dot{C_I} &= PA(C_{Bo} - C_I) - r_T \end{aligned}$$

where V_B is the capillary blood volume, V_I is the interstitial fluid volume, Q_B is the volumetric blood flow rate, PA is the permeability-area product, C_{Bi} is the arterial blood solute concentration, C_{Bo} is the capillary (and venous) blood solute concentration, C_I is the interstitial fluid solute concentration, r_{RBC} is the rate of red blood cell uptake of solute, and r_T is the tissue cellular removal of solute through cell membrane. In the first equation above, the first term on the right side is the effect of convection, the second term corresponds to diffusion, and the last one is the metabolic sink.

Discrete component

Mathematical models developed by biologists are often continuous dynamical systems, as exemplified by much of the work in systems biology. We argue that it is useful to consider hybrid discrete-continuous models to enable more complete, deep, and scalable analysis. Hybrid modeling and analysis can provide great leverage in the realm of complex biological processes, and can also provide abstractions useful in presenting results to human users. The discrete dynamics can arise in many different ways and we discuss some of them below.

The purely continuous models of biological systems can be too large and complex to be maximally useful for simulation and analysis. On the other hand, a fully discrete approximation of the model can sometimes lose crucial and pertinent information. Hybrid systems provide a rigorous foundation for modeling biological systems at desired levels of abstraction, approximation, and simplification. For example, systems that exhibit multiscale dynamics can be simplified by replacing certain slowly changing variables by their piecewise constant approximation. This is particularly useful when the property of interest is defined on a small time scale. Additionally, sigmoidal nonlinearities are commonly observed in biological data correlation, and the corresponding models often use (continuous) sigmoidal functions. These can also be approximated by discrete transitions between piecewise-linear regions. Figure 2 shows a generic plot of data points and the corresponding sigmoidal curve (solid line) generated by tuning parametric sigmoidal curves. The solid curve is chosen to best match the available data, and the heavy dashed line is a piecewise-linear approximation of the data points. The light dotted lines represent nondeterministic bounds on behavior. In some instances, nondeterministic upper and lower bounds are *more* useful than deterministic approximations, because they capture *all* the behaviors of the system.

For example, gene transcription and translation lead to production of proteins in cells. The rate of transcription of the corresponding gene determines the source term in Equation 1 for concentration of that protein. This term is, in general, a function of the concentrations of several other molecules that affect the transcription of the relevant gene. This influence of concentrations of proteins and sigma factors on transcription is conventionally modeled using nonlinear continuous functions. This function is usually a steep sigmoidal curve, which is described using higher-order polynomial expressions or hyperbolic trigonometric functions [3, 23, 26]. Sigmoidal nonlinearities are also observed in many other biological data. For instance, in the case of glucose metabolism in human body, the normalized rate of peripheral glucose uptake (a sink term) is such a nonlinear function of the normalized peripheral interstitial insulin concentration [31].

The use of sigmoidal functions in biological models can be replaced by piecewise constant or piecewise linear approximations as shown by the dashed line in Figure 2, resulting in a hybrid model with a discrete mode change logic [10, 1]. A very steep sigmoidal curve can be approximated by a step function. In the gene regulation example, this corresponds to assuming that a particular gene can be in one of two states: “on” or “off”. A discrete transition describes how

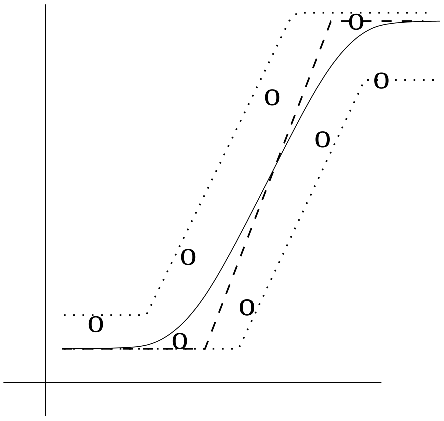

Fig. 2. A generic plot of data points and its various approximations.

the various regulators combine to choose one of these two states. In completely qualitative modeling, one can represent such states with Boolean variables. More refined, but still discrete, stepwise models can result from distinguishing more than two states for genes–for example, "off', "low", "medium" and "high". More complicated discrete logic would then describe the process of choosing between these four possible modes. More accurate approximations for sigmoidal curves are obtained by piecewise linear approximations.

A second source of discrete behavior in models of biological systems is the presence of an inherently discrete process. The physical laws that yield differential equation (continuous) models are applicable only under certain assumptions. For example, the law of mass action holds only when there are large numbers of molecules that are homogeneously mixed. But these assumptions may not be true always. Inside a cell, there are dynamics that are governed by the action of only a few molecules [25]. Ignoring the stochastic aspect temporarily, chemical dynamics at small numbers are best modeled using discrete transitions, cf. the Master equation [11, 7]. This again results in hybrid models of biological processes.

Discrete mode changes can also result from the modeling of faulty modes. In the case of glucose metabolism, the kidney does not excrete any glucose in normal conditions, but it starts excreting glucose if the level of glucose rises very high. This effect can be captured using a discrete transition.

Nondeterminism and analysis of safety properties

Uncertainties and stochastic behavior are common in biology. Rate constants and several other parameters in models of biological systems are determined using algorithms for determining minimal error curve fits for available data points. For example, the rate constants k_j and k_{-j} in Equation 1 and the source and sink terms in that equation are determined in this way. Parameter values thus obtained are “representative” because they do not capture all observed behaviors. The actual value of the parameter is possibly stochastic in a given range. In many cases, we are interested in knowing about *all possible behaviors* of the system, rather than the behavior of the system assuming a representative value for the parameters. For example, when studying the effect of insulin injections on blood glucose concentrations, we wish to know all possible blood glucose concentrations that a human body may exhibit. In such cases uncertainties can be modeled using nondeterminism and the resulting model can be analyzed for all possible behaviors. In Figure 2 the given data points lie between the two piecewise-linear curves shown by dotted lines. The nondeterministic hybrid system resulting from using the two dotted lines as the approximation captures *all* the observed behaviors of the system (and possibly more).

Unknown rate constants can be modeled using unspecified symbolic constants, called parameters, in a hybrid formalism. However, to generate nontrivial models that exhibit interesting behaviors, these parameters need to be constrained to take only certain values. Such constraints can be specified in the model using inequalities over expressions containing these parameters. This gives rise to a highly nondeterministic model, that is, the model can exhibit several different behaviors—one corresponding to every exact numerical instantiation for the parameters that is consistent with the constraints. Although this process of nondeterministic modeling does not accurately capture the stochastic nature of biological processes that arises due to random fluctuations on the small numbers of molecules involved [24], our analysis approach reintroduces some noise by assuming that the unknown parameters are allowed to change randomly (while still remaining consistent with the constraints) finitely many times. Of course, information about exact probabilities remains missing from our nondeterministic model, so results of analysis can sometimes be coarse.

Composition

Compositionality is an important feature required to model and analyze large models of any system. Larger systems are described by putting together smaller networks and component subsystems. Compositional modules are subsystems of a larger system that exhibit identifiable interfaces, are modifiable independently, and enable abstract modeling. Modularity is one of the crucial aspects of designing (and describing) large systems, including computer software and hardware systems. It permits clean and scalable description, and also helps appropriately designed tools in performing simulation and analysis on the models.

It is increasingly apparent that biological systems exhibit certain kinds of clean modularity [4, 5]. Biological examples of modular construction include the universal genetic code, translation into amino acid sequences, protein domains, operon structure, bilipid layer membranes, organelles, organs, communities of organisms, and the one of most interest presently, signaling and metabolic pathways. Cells contain many different regulatory pathways, or networks of interacting proteins or other molecules, in several physical compartments, which interact with each other at certain well-defined points. That is, pathways have been identified that have identifiable interfaces with other pathways, appear to be modifiable independently, and enable abstract modeling. The complete behavior of some aspect of a cell can thus be described by putting together all the various models for the individual pathways and sharing the information on molecular species that are shared by two or more such subsystems.

3 HybridSAL

HybridSAL is a prototype system for hybrid system modeling and analysis [16]. Models of hybrid systems can be written in the HybridSAL language. These models can then be analyzed for safety properties, that is, properties regarding *all* possible behaviors of the system. The analysis is done using an abstraction and model checking framework [35]. This tool has been used on examples from a diverse range of application areas such as automobile transmissions, cruise control algorithms, collision avoidance, and genetic and biochemical networks [9].

HybridSAL can be used to compositionally build parametric hybrid models of biological processes. Some of the general principles described above for generating approximate hybrid models from continuous models built by biologists have not been automated in HybridSAL. The user is responsible for building the appropriate hybrid models of the biological system. In particular, simplifiers and translators need to be added to the existing HybridSAL tool to make it easier to use for biological applications. However, initial translators exist to map SBML models into SAL through BioSPICE.

Analysis

A modeling formalism is only as useful as the analysis tools that support it. The parametric hybrid modeling formalism enables the development of a variety of analysis tools. Combining discrete and continuous modeling techniques results in simpler and more composable models. Compositionality allows for the development of scalable tools. Parametric modeling languages permit the use of tools for model refinement. Presently, we have analysis techniques for (a) automatically creating sound approximations of the model that are smaller and simpler, thus amenable to more intensive (computationally complex) analysis [35], (b) proving properties, such as stability, for the model [9], (c) generating potentially interesting behaviors of the model, and (d) generating constraints on the unknown parameters automatically so that the constrained model exhibits a certain behavior [37]. In the future, we plan to also have tools for model refinement, simplification, and simulation. We also will improve methods of presenting abstract models and the results of hybrid analysis to biological domain experts.

The process of creating sound abstractions is based on combining qualitative techniques [20] with predicate abstraction [12]. It is powered by powerful symbolic reasoning engines [33]. The simplified model generated is a discrete finite-state transition system. It is an abstraction, in a very precise and rigorous sense, of the original model. The abstraction technique can be further optimized for linear [34] and nonlinear systems [36]. The second step of exploration on this finite-state system is carried out using model checking.

4 Examples

We discuss aspects of hybrid modeling and analysis as applied to two specific biological examples.

Glucose metabolism in humans

We will use the human glucose-insulin system and the model of this system proposed by Guyton et al. [13] and Sorensen [31] as an illustrative example. This model has been used to design a model-based predictive control algorithm to maintain normoglycemia, via a closed-loop insulin infusion pump, in the Type I diabetic patient [27]. A formal correctness analysis of any such control algorithm can be established by showing that blood glucose level remains between 70 and 100 mg/dl *always*. For "representative" parameter values, this can perhaps be shown using simulations, but that analysis would never yield real guarantees, since parameter values vary over ranges across different individuals. Thus, higher assurance of bounds on behavior requires analysis over all behaviors of the corresponding nondeterministic model. That is, we suggest that complete exploration of *all* behaviors of an abstracted system provides valuable insight beyond the partial exploration of *some* behaviors (e.g., forward simulation) of a more concrete system model.

The final glucose metabolism model consists of 22 simultaneous nonlinear ordinary differential equations [31]. It is obtained by dividing the human body into six physiologic compartments: brain, heart and lungs, periphery, gut, liver, and kidney. There is a state variable for the glucose and insulin concentration in each of these six compartments. Wherever necessary, these compartments are subdivided into interstitial fluid space and vascular blood space. This model is decomposed into three components in HybridSAL, describing glucose metabolism, insulin metabolism, and glucagon metabolism respectively. Additionally, all nonlinearities in the model arise from sigmoidal functions, which can be eliminated in favor of piecewise linear approximations to yield a hybrid system with linear continuous dynamics. Further simplifications are possible by noticing that the change in glucagon concentrations is very minimal and slow compared to other state variables. The insulin concentrations act as inputs to the glucose module and consequently the insulin concentrations stabilize first, followed by the glucose concentration stabilizing.

The insulin metabolism model has two sources of insulin: pancreatic insulin release and insulin injections. If we set these inputs to zero (say, to model a diabetic patient), then the insulin model stabilizes at 0 because there is no other source of insulin in the model. If we assume that the inputs to the insulin module change very slowly compared to the dynamics of insulin concentration, then we can analyze the system assuming constant inputs. The resulting insulin model is a linear system with one complex eigenvalue with negative real part, and all other eigenvalues are real and negative. This indicates that the system is stable, though it could exhibit some damped oscillation. Using the results to compute approximate reachability sets of linear systems [34], we can easily compute over-approximations of reach sets for this system. The reach sets enable computation of a bound on the insulin concentrations. The glucose metabolism module reduces to a linear system if the insulin inputs are fixed to their lower or upper bounds. The resulting linear system also has one complex and seven negative real eigenvalues. Again using the techniques from [34], we can compute approximate reach sets that bound the modeled behavior of glucose concentrations. Note that because of the construction of the abstractions and approximations, the bounds thus obtained are conservative and robust to small changes in parameter values.

B.Subtilis sporulation initiation

The bacteria Bacillus subtilis initiates spore formation when there is a nutrient deficiency and the environment is not conducive to growth. The cellular commitment to sporulate is regulated by the complex network of transcriptional control of various genes and interactions between various proteins. Based on the data provided to us [39, 38], we constructed a model of the sporulation initiation network of B.Subtilis. The HybridSAL model consists of six components. The phosphorelay chain is described in one of the important components. The effect of promoters and inhibitors on gene regulation was captured via discrete transitions. Unknown rate constants were modeled using parameters. The parameters were constrained by inequalities. In some cases, the constraints were generated using a tool for doing quantifier-elimination over the theory of reals, called QEPCAD [14]. As noted above, the constrained model is highly nondeterministic and it captures a whole spectrum of behaviors.

The constrained parametric hybrid model of the sporulation initiation network was analyzed using hybrid abstraction [35] and model checking for stability properties. The stability properties of the resulting hybrid model were observed to be highly sensitive to the discrete logic modeling gene regulation [36]. The hybrid abstraction approach is partly based on qualitative methods. The analysis of the system using these techniques partially accounts for some stochastic behaviors where the unknown parameters are allowed to fluctuate finitely many times to values consistent with the constraints. This results in several unexpected and interesting behaviors of the sporulation model.

5 Conclusion

The goal of Symbolic Systems Biology is the construction and experimental validation of models and analyses that explain and predict the behavior of biological systems. Symbolic Systems Biology is characterized by a synergistic integration of theory, computation, and experiment. Only through such an interdisciplinary approach can we achieve a scalable, rigorous, and systematic understanding of complex biological processes. Hybrid discrete-continuous formalisms such as those presented at this workshop can be used to provide access to computational analysis enabling accurate modeling of some of the dynamics of biological systems. Together with increasing access to biological network information (through exponentially growing databases and BioSPICE and related tool platforms) and qualitative modeling and analysis techniques, hybrid modeling and analysis of the computation and control of cells, tissues, and organisms may enable Symbolic Systems Biology to begin to be useful to biologists.

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