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Model Identification: A Key Challenge is Computational Systems Biology

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Abstract The primary goal of computational systems biology is the integration of biological data into mathematical models. Due to rapid advances in biological techniques, these data consist more and more of cellular responses in the form of time series measurements of gene expression, protein abundances, or metabolite concentrations following some stimulus. Time series data contain enormous information, but this information is not always explicit but has to be extracted with computational methods. This "inverse" task faces distinct challenges. Most often discussed are purely computational difficulties. Foremost, the algorithms employed for optimizing the fit between model and data often do not converge, converge very slowly or approach a local minimum that is much inferior to the true, global optimum. Other rather evident challenges are related directly to the data, which may be overly noisy, uncertain or partially missing. Less attention has been paid to issues associated with the particular choice of a mathematical model representation, and there has almost been no discussion of the quality of data fit beyond the residual error and the efficiency of an algorithm in terms of the time required to find a satisfactory solution. Finally, there are uncounted statistical questions regarding the design of time series experiments and the assessment of model fits, most of which still await the development of new methods. This presentation discusses inverse tasks in the context of metabolic pathways and describes some advances toward a set of effective algorithms.

Keywords Biochemical Systems Theory (BST); Canonical Model; Metabolic Pathway; Parameter Estimation; Systems Analysis; System Identification

1 Introduction

Systems biology consists of three equally important subspecialties. The first is experimental systems biology, which has set its goal on comprehensively quantifying biological systems, especially through high-density and high-throughput data acquisition at the genomic, proteomic, and metabolomic levels of biological organization. The second subspecialty is engineering, which renders such experimentation possible. Without significant advances in electronics, robotics, sensing, diagnosing, miniaturization and visualization technologies most of the advances of experimental systems biology would not have been possible. The third subspecialty is mathematical and computational systems biology. Here, the enormous amounts of experimental data are collected, organized, and merged into integrative models that capture the functionality of small and large systems in biology. The field of systems biology is on one hand young, for instance, if judged

by scientific citations or Google hits of the term, but on the other hand, biologists have always been intrigued by the multitude of components in natural systems and their coordinated functioning (*e.g.*, [1; 2; 3]). In spite of its young age, systems biology has already moved into a central position of biological thinking and become a mainstream research endeavor. In fact, it has by now become close to impossible to review the entire field in any depth.

In this presentation, I will focus on two related, crucial aspects of computational systems biology, namely the choice of suitable models for biological systems and the identification of their parameter values and structural features. While many of the following comments are relatively general, I will select illustration examples from the area of metabolic pathway analysis. As a first step, let us review the specific role of mathematical and computational models in systems biology.

Modern experiments in molecular biology are capable of generating thousands of data points in a very short period of time. The best-known example is probably a microarray or gene chip that measures the up- or down-regulation of tens of thousands of genes in a single experiment. It is simply not desirable or even feasible to keep track of this many results without computational assistance. Beyond simple bookkeeping, a major task is the interpretation of the results. Can we find patterns in the up- and down-regulation patterns? Are there particular groups of either up- or down-regulated genes that stand out among the thousands of data points? Even without experimental noise, such a question would be difficult to answer, but it becomes much harder in a realistic situation where variability among cells or individuals is intermingled with experimental inaccuracies. Once we identify specific groups of genes that seem to show similar regulation patterns, maybe in an experiment that measures expression at several time points, how should we interpret these groups? Are these genes all controlled by the same transcription factors? Do they code for enzymes in the same pathway? In some cases the answers may be obtained by looking hard at the data, but in more complicated cases, methods of statistics, mathematics and computation are needed to grasp complex relationships, especially if they change over time.

Ultimately the most important role of mathematical and computational analysis may be the construction of models that explain what we observe as well as provide rationale for why we do not observe a different pattern or response. For instance, there seems to be an unwritten rule that feedback in a linear pathway is exerted onto the first step by the final product. Why is this pattern so prevalent? The search and rationalization of such "design principles" can usually not be accomplished with intuitive arguments alone (*e.g.*, [4; 5; 6; 7; 8; 9]). Instead, it is necessary to develop objective answers by comparing two mathematical models that are equal in all aspects except for one feature of interest. In the case of feedback systems one compares a model with feedback from the end product onto the first step with a model that exhibits a different feedback pattern or no feedback at all. The two models are subjected to objective (mathematical) tests and compared with respect to their performance. For instance, a system should respond quickly to a changed environment. Which of the two models performs better in this regard? Which model is more robust? Performing a list of such experiments ultimately shows the advantages and drawbacks of each design, and the modeler has objective means for declaring one model superior to the other, at least in a given set of situations.

A good model is an absolute prerequisite for extrapolations, manipulations, and opti-

mization tasks. Examples abound. For instance, imagine that a microorganism naturally generates small amounts of some organic compound that is of interest either as a bulk product, such as biofuel, or as a molecule of high purity, such as insulin or some amino acid that is valuable because it is used in foods or medications. A typical goal in metabolic engineering is to manipulate the microorganism with genetic methods in such a way that it generates the product of interest in much higher yield than the wild type. This task has been approached in the past with the introduction of random mutations and the selection of strains that showed improved performance. However, over time this method tends to become less and less successful, because the likelihood decreases that better solutions can be found by chance mutations. Given a reliable model of the cell, one would be able to predict which specific combinations of alterations in enzyme activities or gene expression would improve, and in the end optimize, yield (*cf.*, [10]). To a limited degree such predictions are possible with today's models. A very similar issue arises in a rather different context, namely the development of new drugs: a comprehensive model of a disease process would be an excellent test bed for screening drug targets and the development of combination drug treatments [11]. In most cases, we have not reached this level of sophistication in biological model design, for manifold reasons. Often not all relevant data are available to set up a reliable model, or it may happen that the data only illuminate a small part of the biochemistry and physiology that we need for understanding a phenomenon sufficiently well to translate it into a model. For example, if all data refer to healthy individuals, even a "perfect" model that represents the system well might not necessarily allow extrapolations to disease states. In most situations, however, the major stumbling blocks to model development are the identification of a suitable model structure and the estimation of optimal parameter values from data. These two tasks are actually closely related to each other, if a convenient model approach is chosen, as will become clear in the following sections.

2 Canonical Representations as Good Defaults for Systems Modeling

The task of setting up a mathematical model of a biological system consists of selecting and representing the essential aspects of the system in the form of equations. The task is complicated for the following reasons. First, linear models, which are very convenient for all kinds of mathematical analyses, are usually not very well suited, because biological phenomena exhibit intrinsically nonlinear behaviors. They always saturate or change dramatically (for instance, get sick or die) if pushed out of their normal operating range. They may show stable oscillations, which are not consistent with linear models. As an example, suppose we attempt to model the human heart beat. As a simple default one could choose a sine oscillator. It is easy to adjust its frequency, amplitude and phase, and there are numerous methods for analysis. However, if the amplitude of this oscillator is perturbed (which could correspond to running up the stairs; arrow in Figure 1), the sine oscillator does not return to its normal beat, but continues to beat with the altered amplitude, whereas the real heart recovers. This ability of recovery is the mathematical hallmark of a limit cycle oscillator, which requires at least one nonlinearity somewhere in the model. In the case of the van der Pol oscillator [12] (shown in Figure 1), the model contains a cubic term.

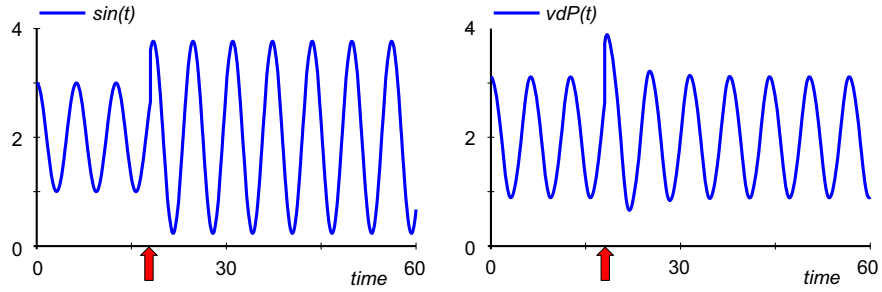

Figure 1: A simple sine oscillator (left) and a limit cycle (right), as proposed by van der Pol in 1928, may look similar in appearance, but show distinctly different responses to perturbations: if there is an external change in amplitude (arrows), the limit cycle oscillator "recovers," while the sine oscillator does not. See Text for additional details.

The failure of linear models to represent some of the observed responses in biology necessitates the search for good nonlinear representations. These can be as complicated as needed, and there is little chance that we would ever run out of options. The challenge is that nature has not provided us with guidelines for how to choose a nonlinear model for a specific biological phenomenon of interest. One might want to begin by looking at physics and employing its proven laws. While biological phenomena of course occur in the physical world, one quickly finds that the physical laws are so deeply embedded in complex biological systems that representations based on these laws would become much too complicated. Much more useful would be higher-level "meta-laws" of biology, but these have not been discovered to date.

A good compromise in the search for representations is the use of *canonical* models. Such models have a fixed mathematical structure, but are still flexible enough to model all types of nonlinearities we might encounter in biology. The two most prominent nonlinear canonical models are Lotka-Volterra (LV) models and power-law (PL) models within the modeling framework of Biochemical Systems Theory (BST). Hundreds of articles and books have been written about these types of models (*e.g.*, [10; 13; 14; 15]). LV models have the format

$$\dot{X}_i = X_i \cdot (a_i + b_{ij} \sum_{j=1}^{n} X_j) \tag{1}$$

where the X_i code for dependent variables, $\dot{X}_i = dX_i/dt$, and a_i and b_{ij} are parameters. LV models have found their richest biological applications in ecology, where they describe the interactions between species [16]. We will not discuss them here further, because they are not as suitable for the representation of molecular and biochemical systems.

The characteristic feature of PL models in BST is the representation of all processes as products of power functions of the type $\gamma \cdot X_1^{f_1} X_2^{f_2} \cdots X_n^{f_n}$. This format is not arbitrary, but the rigorous result of linearization of the process in a logarithmic space [17; 18]. Canonical PL models come in two significant variations. The first is the Generalized Mass Action (GMA) form, in which every process is individually represented by a product of power functions. The alternative is the S-system form, where all process leading to the

same node (variable) are taken together and collectively represented by one product of power functions, and the same is done for all processes leaving a node. It might be best to demonstrate the two strategies with a concrete example (Figure 2).

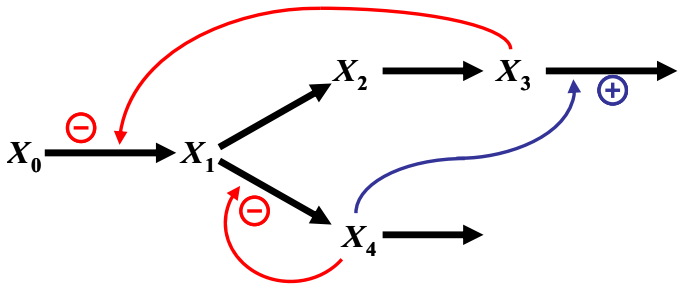

Figure 2: Illustration pathway with one branch point, two feedback inhibition signals and one activating signal.

The GMA and S-system formats lead to the same PL representation except at branch points, like the degradation of X_1 toward X_2 and X_4. As a common example for both forms, the production of X_1 in the pathway of Figure 2 is affected directly by the external (independent, constant) variable X_0 and also by the dependent variable X_3 via feedback inhibition. The production of X_1 is therefore represented as $\alpha_1 X_0^{g10} X_3^{g13}$. Here we have introduced some notation, quasi on the fly. Namely, those and only those variables directly affecting a process are included, and each of them receives an exponent, called a *kinetic order*. If the variable contributes to an increase (decrease) in the magnitude of the process, the real-valued kinetic order g is positive (negative). If the variable has no effect, the kinetic order is zero, thereby de facto eliminating the variable from the term. The term furthermore contains a non-negative *rate constant* α that quantifies the turn-over rate of the process. The representations for the production and degradation of X_2, X_3, and X_4 are constructed in the same fashion. The only difference between the GMA and S-formats occurs in the degradation of X_1, which consists of two processes. In the GMA format, the result consists of two power-law terms, while the S-system format contains only one. With typical numerical values for all parameters, the systems might read as follows:

$$
\begin{array}{lll}
\text{GMA} & \dot{X}_1 = 20X_0X_3^{-0.9} - 8X_1^{0.75} - 12X_1^{0.5}X_4^{-1} & X_1(t_0) = 0.8 \\
\text{S-system:} & \dot{X}_1 = 20X_0X_3^{-0.9} - 19X_1^{0.64}X_4^{-0.45} & X_1(t_0) = 0.8 \\
\hline
\text{GMA / S:} & \dot{X}_2 = 8X_1^{0.75} - 5X_2^{0.3} & X_2(t_0) = 1 \\
\text{GMA / S:} & \dot{X}_3 = 5X_2^{0.3} - 5X_3^{0.5}X_4^{0.2} & X_3(t_0) = 0.5 \\
\text{GMA / S:} & \dot{X}_4 = 12X_1^{0.5}X_4^{-1} - 4X_4^{0.8} & X_4(t_0) = 6 \\
\text{GMA / S:} & X_0 = 1.1 \text{ (constant)} &
\end{array}
\qquad (2)
$$

Figure 3 shows a typical simulation result. The graph demonstrates that the two systems produce slightly, but not very, different responses. This similarity is seen in many cases, but not always. The conversion of a GMA into the corresponding S-system model is a matter of straightforward computation (*e.g.*, [15]).

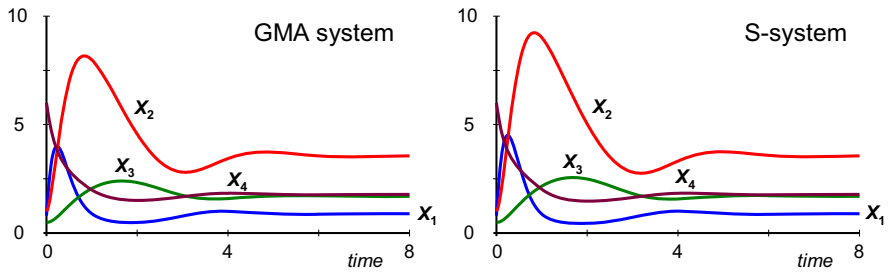

Figure 3: Results of a simulation with the GMA and S-system models in Eq. (2). The responses are similar, but not identical.

Canonical models have a number of advantages. These begin immediately at the model design step, where the rules of BST prescribe how to set up the equations. Namely, as we have seen with the example above, each dependent variable is represented with a differential equation that contains as many terms as processes affecting the variable (GMA form) or with at most one production and one degradation term each (S-system form). Each term in the equations consists of a product of power-law functions that contains exactly those variables that have a direct effect on the modeled process. This simple recipe for model design has another important consequence: The structure of the pathway is mapped one-to-one onto the corresponding power-law model. This implies the following observations. First, writing the equations is straightforward and can be accomplished with a computer program. Second, if it is our task to identify the flux and regulation structure of a model with n dependent variables from experimental data, we can in principle begin with the most general S-system model with n equations and each variable permitted in every term, thereby allowing for the possibility that any kinetic order could be non-zero (*e.g.*, [19]). Biologically speaking this would mean that any variable could potentially affect any process in a direct fashion. Applying a fitting algorithm to experimental data that are measured at several time points (see later section), the result would be a set of numerical values for all parameter values. Typically, many of the kinetic orders would be very close to zero, which we would interpret as the fact that the corresponding variables would indeed ***not*** affect the given term. For instance, the result $g_{12} = 0$ would mean that, even though we originally allowed for the possibility that X_2 could directly affect the production of X_1, the data indicated that this effect is negligible. The deduction from this discussion is that the identification of the structure and regulation of a power-law model from experimental time series data is reduced in difficulty to the simpler task of estimating parameter values. This reduction is a very significant simplification: just imagine finding parameter values in a model (or a list of models) whose structure is not known. Other advantages of canonical models include the fact that algorithms can be developed specifically for these types of models, which permits optimization of efficiency. For instance, Irvine and Savageau [20] showed that BST models are very efficiently solved numerically with a customized Taylor series method. This method is the basis for effective software [21; 22] and can also be applied to features like time dependent sensitivities [23; 24; 25]. The power-law form is also well suited for steady-state optimization, for instance, in metabolic engineering.

3 Challenges of Parameter Estimation and Model Identification

Among all steps in the modeling process, parameter estimation is the most difficult. Methods for parameter estimation are directly dependent on the type of available data. For a long time, the data supporting metabolic models consisted almost exclusively of characteristics of enzymes that catalyze the conversions of metabolites into other metabolites and are therefore the drivers of pathway systems. The generic strategy for model construction in this situation has been to transform the measured characteristics into parameters of individual process descriptions and to merge all process descriptions into a comprehensive model. This process is typically very cumbersome (*e.g.*, [26]) and often fails when the model is subsequently tested against new data. It is therefore almost always necessary to revise the model or the estimated parameter in an iterative fashion that may take months or years.

A distinctly different method of estimation is becoming increasingly more popular, due to modern methods of molecular biology. Specifically, it is now possible to measure the responses of cells to a stimulus at several successive time points. At least in principle, these time series permit algorithmic searches for parameter values that make the model exhibit the same dynamics as the data. The challenges of this type of model identification, or the simpler task of estimating parameter values, fall into five categories. The first class of issues is related to the choice of a model. Without a canonical form, this task is difficult and often rather biased. Imagine having to explore an unknown number of alternatives for possible model formats. Even within the limited area of enzyme kinetics, one would have to work with a variety of candidate models, including irreversible and reversible Michaelis-Menten rate laws, Hill functions, rate laws subjected to various modulations, such as competitive, non-competitive, allosteric, or mixed inhibition, as well as more complex formulations for ping-pong, bi-bi, and other types of mechanisms, as detailed in Schultz [27]. If we use a canonical form, this issue is incomparably easier to tackle. Nonetheless, there are still significant obstacles to overcome. For instance, it may be that several canonical models fit the data, raising the question of which model is "correct" or at least superior to other candidates. If the competing models come with about the same residual error, it is not trivial to decide objectively which model should be chosen.

The second class of challenges is related to fitting algorithms. At first glance it may seem that the problem has been solved once and for all, because software packages like MatLab or Mathematica contain many options. The oldest algorithms are implementations of nonlinear regression schemes, which conceptually go back to Newton's methods and use hill climbing and interpolation techniques. Newer methods include genetic algorithms and simulated annealing, as well as lesser known techniques such as swarm and ant colony methods. Although many options are available in the literature, none of them has proven effective in the majority of systems analyzed. Reasons are plentiful. Realistic systems models are nonlinear and allow many local minima, which here translate into particular sets of parameter values that are better than other values nearby but worse than the truly optimal solution. The models are almost always dynamic and therefore require the numerical solution of the describing differential equations. This numerical integration easily uses in excess of 95% of the total computation time and often leads to algorithmic failure or non-convergence to a suitable solution [19]. As a partial remedy, it is possible

to estimate the slopes of all time courses at many time points and to substitute these estimates for the derivatives of the differential equations [19; 28]. The result is a conversion of each differential equation of the system into a set of many algebraic equations. The advantage of this strategy is that integration of differential equations is no longer needed. Furthermore, the strategy decouples the system of differential equations so that each equation can be addressed separately or in parallel. Essentially all recent papers on parameter estimation in canonical models have made use of this strategy. Nevertheless, the computational problems are not yet solved, and there is a great need for effective, scalable estimation methods. A step in the right direction for BST models may be a method called Alternating Regression [29] and its extension of Eigenvector Optimization [30], which make full use of the specific structure of power-law methods.

The third challenge to fitting time series data derives from the data themselves. As it to be expected, biological data contain noise, replicate experimental results are often quite different, sometimes data, or even whole time courses, are missing, and in some cases the experimenter does not even know that relevant variables have not been measured even though they affect the system. In addition, it may happen that the data are not informative or that time courses within the same dataset are related in a fashion that complicates the estimation. Specific statistical techniques will be needed to address these issues.

The fifth class of challenges is related to the quality of the model fit beyond the residual error. Very few articles have discussed this issue. The situation leading to this challenge is quite typical. A model has been estimated from data and is now used to make predictions of responses under altered experimental conditions. However, even though the model represented the test data with sufficient accuracy, the model predictions are unacceptably inaccurate. A frequent reason for this failure is that the original estimation had resulted in one of many possible models and happened to be suboptimal, in spite of an acceptable residual error. This apparent contradiction is possible if there is error compensation between different terms. For instance, it could be that both the production and degradation of a variable are largely overestimated, but the result is a similar net value of the dynamics of the variable. However, if extrapolations are done with this model, for instance, by altering the input to the system, it may happen that the compensation between the two terms no longer holds. As an example, let's revisit the example in Eq. (2). Suppose the rate constants in all equations are estimated within 10% of the true values, but both production and degradation term of variable X_2 were somehow estimated to include the multiplicative term $0.2X_2$. While this appears to be a rather significant change, which would be interpreted as X_2 activating its own production, the time courses (lines in Fig. 4a) are surprisingly similar to the original trends (dots in Fig. 4a), and one might conclude that the representation is sufficiently accurate. Now suppose the next task is to predict the outcome of an experiment where the input to the system is doubled. It is easy to run a simulation with both models to test the reliability of any predictions. The result is a very noticeable difference between the "true" model and the alternative model in which the two terms in the second equation are mis-estimated (Fig. 4b). We recently proposed a methodological framework, called Dynamic Flux Estimation (DFE), which avoids compensation between terms to some degree [31]. DFE consists of two phases. The first phase is model free and targets solely the stoichiometry of the system. In other words, it assures that the fluxes are balanced for all variables at all time points. The result of this phase is a model free (numerical or graphical) representation of each flux as a function of

all contributing variables. In the second phase of DFE, the flux representations are fitted with suitable models, such as power-law or Michaelis-Menten functions. DFE is more robust toward compensation between terms and therefore less vulnerable to problems in extrapolations. DFE is also a good means of combining time series estimation with the more traditional estimation of individual processes. The main drawback of DFE is its reliance on rich time series data sets, which are not always available.

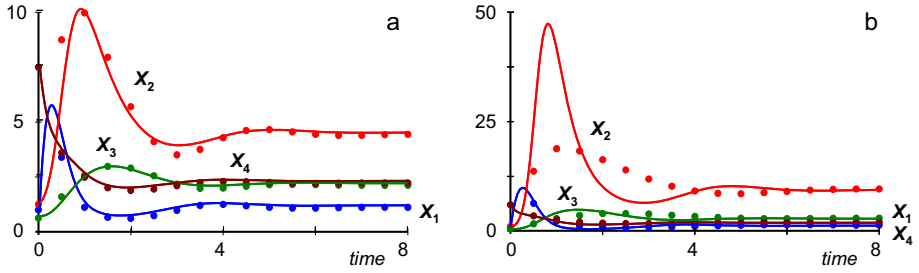

Figure 4: Although the parameter values of a model may have been estimated quite inaccurately, the fit to data may be satisfactory, as shown in panel (a) where "data" (dots) obtained from the model in Eq. (2) were fitted with a different set of parameter values (lines). However, if the two models are extrapolated to different conditions (doubled input), their responses may be quite different (panel b; dots and lines corresponding to models in panel a).

Finally, the fifth class of challenges in model estimation is of a statistical nature and generically asks how to design good time series experiments and how significant the estimation results are. The literature on this challenge is scarce, and solid statistical work will be needed in the future. During the model design phase, a crucial question is how many data points should be measured and how many replicates are necessary. Clearly, biological experiments can be expensive, so that there will often be a trade-off between data points and replicates. The number of needed time points evidently depends on the complexity of the time courses. If these are simple shoulder curves, a few points might be sufficient. By contrast, highly fluctuating time trends will require more measurements for a reliable characterization, and the number increases if the data are noisy. At this point, there is no good measure for the complexity of a time course and the number of data points its quantification requires. In some cases, the model identification leads to alternative models. Especially if these contain different numbers of non-zero parameters, a statistical measure for the quality of fit is needed. One would expect that models with higher numbers of parameters fit the data better than simpler models. For linear models, appropriate measures for such an assessment are available, but the corresponding measures for nonlinear models still need to be worked out. Another question in the context of two candidate models asks what additional experiments would optimally distinguish between the models and identify one as superior to the other. These questions await the development of refined statistical techniques for dynamical systems.

4 Conclusions

The 21^{st} Century is widely regarded as the century of biology. The technical means for testing and analyzing biological systems are unprecedented, and doing modern biology is a truly exciting endeavor. Accompanying the experimental advances must be computational methods that aid in the bookkeeping of the flood of data that is generated by laboratories around the world and that help us integrate data with their context into higher-level information and, ultimately, a deeper understanding of how biological systems work and what their design and operating principles are. At the center of this effort is almost always a mathematical model that allows investigations not possible in a wet experiment. This presentation has discussed issues of choosing a suitable model and of identifying its structure and regulation. If a canonical representation is chosen to model a biological system, the identification task becomes a task of parameter estimation, which is much simpler, yet still presents severe computational challenges. We have made significant progress toward efficient solutions of the parameter estimation task, but much more needs to be done in the future.

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